EMBRYO DEVELOPMENT IN NICOTIANA SPECIES HYBRIDS

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INTRODUCTION

In a study of the compatibilities of certain Nicotiana species, several crosses were reported which produce seeds unable to germinate. (McCRAY 1932). Many others are recorded in the literature on this genus. Some of these seeds are parthenocarpic; others have embryos not able to develop. In still other cases the hybrid germinates but is unable to carry on further. All degrees of vigor have been observed from these conditions up to heterosis. Several interesting questions might be raised in connection with the development of the hybrid embryos displaying such different degrees of viability and vigor. Does the hybrid begin its development as soon after pollination as does the new individual of pure species? That is to say, does fertilization take place as readily in one case as in the other? Do the cell divisions in the embryo and its consequent growth proceed as rapidly in the hybrid as in the pure species? If so, when does growth in the non-viable embryos stop? If not, what sort of growth rate does the hybrid have as compared with its pure parent? These are the questions which the present study attempts to answer.

Strangely, considering all the work which biologists, especially botanists, have done on hybridization, the scientific literature sheds very little light on these problems. It is nearly an unworked field. So far as I have been able to find only two studies have any bearing on the problem in hand, those of KOSTOFF (1930) and BRIEGER (1928), both on Nicotiana.

To be sure KRUMHOLZ (1930) compared the size of embryos of interspecific Oenothera hybrids with that of embryos of the pure species, but he measured only the fully matured embryos, and says nothing about their growth. Incidentally it might be noted here that he found, in reciprocal crosses, the embryo size to be practically determined by the female used in the cross. If the female was of a large seeded species, the hybrid embryo was large and *vice versa*.

KOSTOFF describes several experiments on the formation and development of hybrid embryos. He points out the fact that in some crosses the embryo develops for a few (up to 15) days, then stops, followed by the death of the ovule; he mentions other cases in which the young hybrid persists for a longer period, although not able to mature to the point where germination is possible. He also observed that parthenocarpic seeds and

those with small embryos are always smaller than seeds resulting from selfing. The sizes of several kinds of interspecific hybrid embryos of Nicotiana are recorded.

On the point of rate of growth this author states only that, with respect to certain crosses, "the embryos begin to develop quite normally, but when compared with the embryos obtained after selfing, a retardation of the growth of the hybrid embryo took place a few days after fertilization. The time of the retardation of the embryonic growth depends, of course, on the cross combination. This retardation gradually increases with the embryonic period of growth."

These observations give a very inadequate picture of the comparative growth rates. On the other hand, however, this investigator made a partial study of causes of failure of the hybrid to grow normally, and of some other effects of the hybrid condition. More specifically, he discusses the formation of extra amounts of starch outside the nucellus, and some other morphological abnormalities in ovules carrying hybrid embryos.

In view of the considerable differences in different Nicotiana hybrids it was thought advisable, in the present study, to choose crosses representing several different degrees of vigor in the hybrid. Accordingly four different groups of cross combinations were recognized and two crosses selected to test the embryo development in each group. Group I includes hybrids which fail in their embryonic development very soon after fertilization; group II comprises others which also fail to complete embryonic growth but develop somewhat farther than those in group I. Group III hybrids grow to their full embryonic size, give a partial germination, but are weak and unable to mature. Those other more successful combinations which do mature constitute group IV.

The crosses to represent the first two groups were chosen largely on the strength of KOSTOFF's data. They are, for group I, *N. rustica*×*N. glauca* and *N. rustica*×*N. longiflora;* for group II, *N. rustica*×*N. Rusbyi* and *N. rustica*×*N. Palmeri*. In each of these crosses the *N. rustica* var. *brasilia* was used.

N. nudicaulis×*N. Tabacum* and *N. suaveolens*×*N. Tabacum* are of group III. CHRISTOFF (1928) reports both of these crosses as being weak, and EAST (1928) and KOSTOFF concur in the observation on the latter. I myself tested them also. Of 100 seeds of *N. nudicaulis*×*N. Tabacum* 7 germinated but died a few days later. Of a later planting of 68 seeds 10 germinated and again died in the cotyledon stage. *N. suaveolens*×*N. Tabacum* gave a strong germination, but 10 plants which were transferred to small pots all died at the same young seedling stage. KOSTOFF tested many seedlings with identical results. In both of these crosses I used the *N. Tabacum* var. *purpurea*.

EAST and KOSTOFF have each grown to maturity plants of *N. Tabacum* × *N. glauca*. *N. paniculata* × *N. glauca* is reported by EAST, and by GOODSPEED and CLAUSEN, as having matured hybrid plants (EAST 1928). These two hybrids, which I also found vigorous in my own tests, represent group IV. In the former of these crosses I used the *N. Tabacum* var. *sanguinea*.

MATERIALS AND METHODS

Because of a possible bearing on the results obtained it is first to be noted that the pollinations for the series of observations to be reported here were made over a period of several months, from October to May. It might have been preferable to make them within a shorter period so that all the embryos would have been subjected to more nearly the same environmental conditions, especially temperature and length of days, but under the circumstances this was impossible. This point will be discussed later.

The plants used were either obtained from Bussey Institution, HARVARD UNIVERSITY, where the work was done, or grown from seed I had saved in the course of earlier experiments on the same species already referred to. All the plants were kept in a screened greenhouse, the pollinations were made by hand, and the usual precautions were taken to prevent accidental crossing.

The capsules were fixed in Allen's modification of Bouin's fluid, B-15, and stained in Haidenhain's haematoxylin.

The developing capsules were fixed at intervals of two days, so the embryo lengths were recorded at 7, 9, 11, 13, 15, 17, 19, and 21 days after pollination. It was thought that by 21 days after pollination the embryos would have practically attained their full size, as the seeds often ripen at about 25 days. This apparently is not quite true as they continued, in some instances, to increase in length rather rapidly up to 21 days, indicating that there would have been still further growth had the capsules not been removed. However the embryos were sufficiently matured by this time to show clearly the difference in development between the hybrid and its maternal parent, which is the point in which we are especially interested.

Because of the desirability of measuring several embryos of each age, the whole capsules were fixed and embedded in paraffin, so that a number of ovules were sectioned and mounted on each slide. Considerable difficulty was encountered in the sectioning from two causes, (1) incompleteness of infiltration of paraffin into the ovules, especially when nearing maturity, and (2) the ease with which the ovules were broken loose from the placenta, and the embryos removed from the ovules. Fortunately the

embryos are quite strong and usually remain intact even when they are pulled out of place by the knife.

To obviate this latter difficulty I experimented with cedar oil as a clearing agent to prevent some of the hardening effects of alcohol and xylol in the process of dehydration and clearing. I also tried ZIRKLE's (1930) butyl alcohol procedure. This seemed to give somewhat better results and was followed in most of this work. KRUMBHOLZ (1930) soaked his Oenothera seeds in water until they were soft and then removed the embryos before fixing. But he was dealing with only the matured seeds which simplified the problem in this regard. A half and half mixture of hard and soft paraffin seemed to facilitate the sectioning somewhat as compared with the soft paraffin.

The embryos were measured with an eyepiece micrometer. This procedure is simple enough, except when the embryos are quite sharply curved, as are the older ones in some instances. In these cases the curved axes of the embryos were divided into a number of short arcs, each one approximating a straight line, and the ocular gradually rotated so the scale was brought into contact with these arcs successively throughout the length of the embryo. Sometimes in the older embryos there was a difference in the measurement depending on which side of the embryo was measured. That is to say the cotyledons were not of the same length. In these cases the average of the two measurements was recorded. Practically all the embryos were measured in such position that the two cotyledons lay side by side at about the same level. In the latter part of the work a few measurements were taken from the "side view" of the embryo, one cotyledon lying beneath the other.

The number of measurements at each age varied from 1 to 20, the average number being 9 2/3. In only 8 cases, however, were less than 5 embryos measured, and in half the cases 10 or more measurements were averaged together. Altogether 761 embryos are included in the data.

OBSERVATIONS AND RESULTS

No fusions of egg and sperm were seen in the course of these observations, but in one preparation of *N. rustica*×*N. glauca* at 7 days and in another of *N. Tabacum* at 7 days, I found fertilized egg cells which had undergone only one division, as shown in figure 1a. In several instances the first measurements recorded were made on single cell embryos. It therefore seems quite clear that fertilization usually took place on the seventh day,—or on the ninth day in two instances. An exception is seen in *N. rustica*. The 7 day embryos in this case are spherical, much larger than the others, and have about 32 cells.

Although not particularly pertinent to the results of the study it is of

some interest to note the changes in shape of the embryo as it develops. There are minor differences in embryo shape in the different species used, but in general the process of development is about as follows. Following

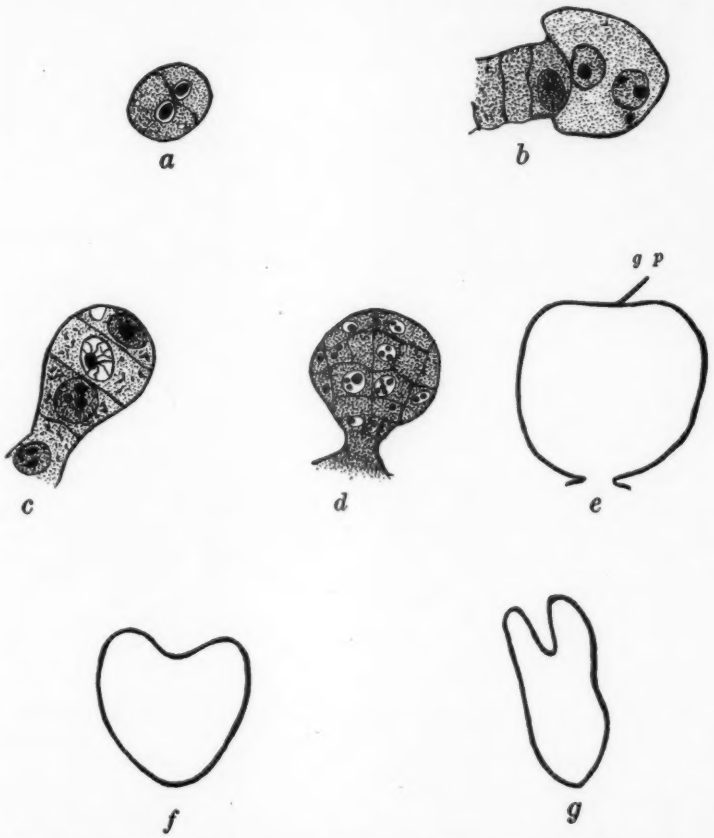

FIGURE 1.—Embryo development in Nicotiana species, e, f and g diagrammatic, showing shape only. a. Fertilized egg cell in first division, *N. rustica*×*N. glauca*, 7 days after pollination (×360). b. Embryo at end of suspensor, containing two nuclei, but cell not yet divided, *N. paniculata*, 7 days (×810). c. *N. suaveolens* embryo in 3 cell stage, 7 days (×360). d. *N. rustica* var. *brasilia* in about 32 cell stage, 7 days (×432). e. *N. rustica* var. *brasilia*, 11 days. g.p., growing point (×432). f. *N. paniculata*, 13 days (×330). g. *N. paniculata*, 15 days (×110).

fertilization the new zygote undergoes several divisions, resulting in a linear series of cells, as is common in Angiosperms. The distal end of the chain is the embryo proper, the others constituting the pro-embryo or suspensor, which attaches the embryo to the micropylar end of the ovule

(figure 1b). The semicircular embryo cell then enters upon its long series of cell divisions which eventually may bring it to maturity. The first divisions are transverse so the linear series of cells is extended (figure 1c). As cell divisions ensue in other planes, the embryo gradually assumes a spherical shape as in figure 1d. Within a few days the distal end of the embryo has widened out so that the longitudinal section presents a wide rounded wedge (figure 1e). By this time the cells have undergone some differentiation, and the growing point is located, as shown in the figure, in the middle portion of the distal end. As the cotyledons begin to appear the two sides of the wedge lengthen until the embryo has a distinctly forked appearance which it retains to maturity (figures 1f and g).

All the average embryo lengths are shown in table 1, in millimeters, and the same results are shown graphically in figures 2 to 6.

TABLE 1

Growth in length of embryos of Nicotiana species and their hybrids (measured in mm).

DAYS FROM POLLINATION	SPECIES OR HYBRID						
	rustica VAR. *brasilia*	*rustica*× *glauca* I	*rustica*× *longiflora* I	*rustica*× *Rusbyi* II	*rustica*× *Palmeri* II	*nudicaulis*	*nudicaulis*× *Tabacum* VAR. *purpurea* III
7	0.0481	0.0209	0.0136	..	0.0416	0.0234	0.0234
9	0.1569	..	..	0.0435	0.0447	0.0446	0.0207
11	0.1214	..	..	0.0370	0.0444	0.0360	0.0333
13	0.2303	..	..	..	0.0574	0.0592	0.0567
15	0.4435	..	..	..	..	0.0576	0.1070
17	0.6375	..	..	..	..	0.1010	0.1551
19	0.8006	..	..	..	..	0.1126	0.0843
21	0.9020	..	..	..	..	0.4572	0.3152

DAYS FROM POLLINATION	SPECIES OR HYBRID					
	suaveolens	*suaveolens*× *Tabacum* VAR. *purpurea* III	*Tabacum* VAR. *sanguinea*	*Tabacum* VAR. *sanguinea*× *glauca* IV	*paniculata*	*paniculata*× *glauca* IV
7	0.0296	0.0281	0.0182	..	0.0210	0.0111
9	0.0390	0.0470	0.0291	0.0185	0.0244	0.0204
11	0.0807	0.0539	0.0412	0.0345	0.0838	0.0616
13	0.0574	0.0662	0.0668	0.0395	0.0903	0.0631
15	0.1194	0.1117	0.2216	0.0812	0.2777	0.0722
17	0.7655	0.2888	0.4880	0.2749	0.3915	0.1389
19	0.6769	0.6383	0.4945	0.3246	0.3930	0.1820
21	0.5765	0.7625	0.5183	0.4087	0.4413	0.2563

The hybrids of group I, *N. rustica*×*N. glauca* and *N. rustica*×*N. longiflora*, made almost no growth at all. Measurements were secured only at 7 days. Then the embryos apparently died and the ovules degenerated. The appearance of such a dead ovule is indicated in figure 7.

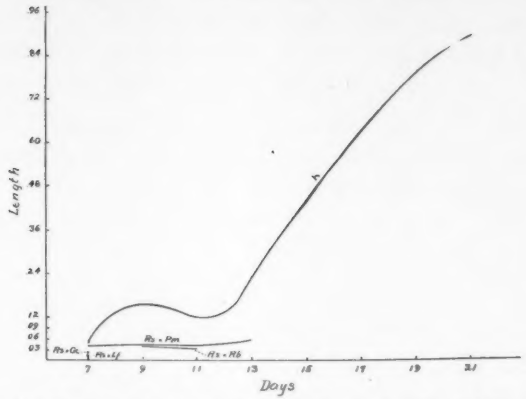

FIGURE 2.—Growth curves of embryos. Rs, *N. rustica* var. *brasilia;* Rs×Gc, *N. rustica*×*N. glauca;* Rs×Lf, *N. rustica*×*N. longiflora;* Rs×Rb, *N. rustica*×*N. Rusbyi;* Rs×Pm, *N. rustica* ×*N. Palmeri.*

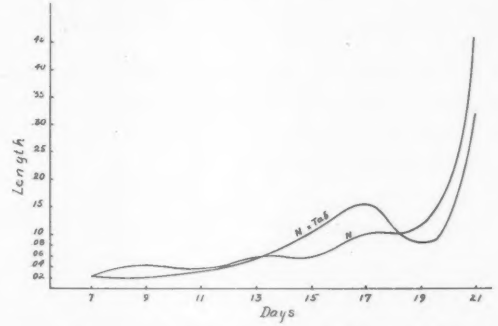

FIGURE 3.—Growth curves of embryos. N, *N. nudicaulis;* N×Tab., *N. nudicaulis*×*N. Tabacum.*

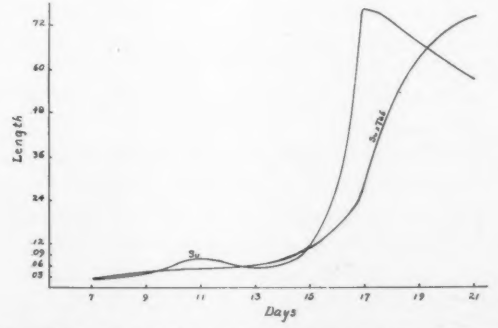

FIGURE 4.—Growth curves of embryos. Su, *N. suaveolens;* Su×Tab, *N. suaveolens*×*N. Tabacum.*

The hybrids of group II persisted somewhat longer, but they also failed to make any appreciable growth. *N. rustica*×*N. Rusbyi* was observed only at 9 and 11 days, and *N. rustica*×*N. Palmeri* died after the thirteenth day (figure 8).

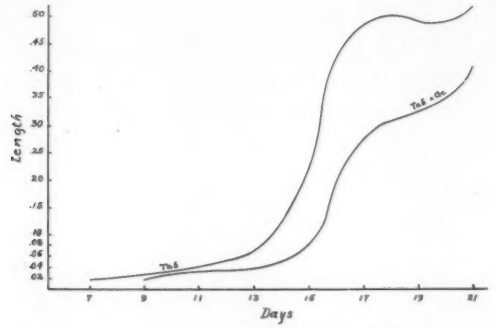

FIGURE 5.—Growth curves of embryos. Tab, *N. Tabacum* var. *sanguinea;* Tab×Gc, *N. Tabacum* var. *sanguinea*×*N. glauca.*

While our chief interest might be in the unsuccessful hybrids of the preceding groups, there is less to say of them than of the others. They seemed doomed from the start. Whatever the nature of the incompatibility exhibited they are not able to grow at all.

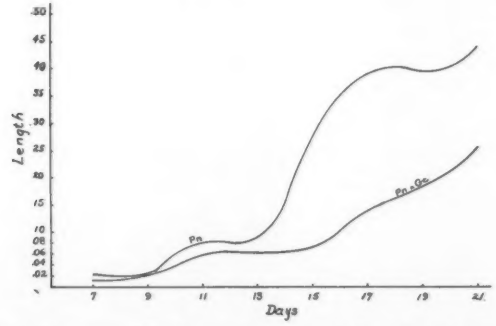

FIGURE 6.—Growth curves of embryos. Pn, *N. paniculata;* Pn×Gc, *N. paniculata*×*N. glauca.*

In all the other crosses the full number of eight observations was made except in *N. Tabacum*×*N. glauca* where also the embryo made its first appearance at 9 days. The progress of the hybrids of groups III and IV can, naturally, best be studied from the growth curves. A general similarity between the curves will at once be apparent, especially if we omit for the present those representing *N. suaveolens* (figure 4), *N. nudicaulis*

and *N. nudicaulis*×*N. Tabacum* (figure 3). These three curves will be discussed presently.

DISCUSSION

PEARL (1925) has brought together data from many sources and shown that many growth curves, based on such different forms of life as rats, pumpkins, and tadpoles, and even curves representing growth in populations of yeast, flies and human beings, assume much the same form. He concludes that wherever growth is based on multiplication of cells a similar logistic, S-shaped curve, first employed by VERHULST (1838) in describing population growth, and since used by ROBERTSON (1923) and others, could be used to represent the growth of the organism in question.

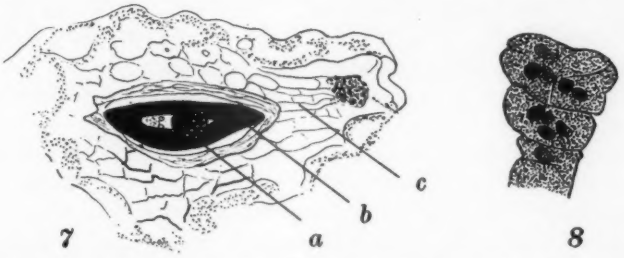

FIGURE 7.—Dead ovule, *N. rustica* var. *brasilia*×*N. glauca*, 11 days after pollination (×432). a. embryo; b. nucellus; c. endosperm.

FIGURE 8.—Embryo of *N. rustica* var. *brasilia*×*N. Rusbyi*, 11 days, in state of disorganization. Note shrunken appearance and larger number of nuclei than of cells (×432).

My six curves which are most alike also approximate this logistic, S-shaped curve. Especially is this true of the one representing *N. suaveolens* ×*N. Tabacum* (figure 4). Apparently the same law, as might be expected, governs growth in the embryonic state as well as in later life.

PEARL also brings out the fact that the complete growth curve of an individual might cover successive cycles of growth, each of which could be represented by such a logistic curve. It seems from the data here presented that embryo development constitutes the first of such cycles, which would be followed, as illustrated by PEARL'S pumpkin curve for example, by other cycles of growth after germination. The curve for *N. paniculata* (figure 6), indeed, suggests the occurrence of two cycles during the embryonic stage alone. But since this curve is an exception in that regard it is assumed that the hump in this curve at 11 days which gives it that appearance is due to the same kind of variations as those which produced a hump at 9 days in *N. rustica* (figure 2). These variations are not considered significant.

The main point for our present study is the comparison between the

hybrids in the different categories, and between each hybrid and its maternal parent species. Again a similarity is noted between the different pairs of curves—leaving out of account all the curves in figure 2 for obvious reasons. The curve for each hybrid approximates that for the mother species, but falls somewhat below the latter. The retardation in the growth rate of the hybrid becomes more noticeable at the time when the increase in growth rate becomes suddenly much greater, in other words, when the curve turns sharply upward. This is at 13 days after pollination in *N. Tabacum* and *N. paniculata* and their hybrids, at 15 days in *N. suaveolens* and *N. suaveolens*×*N. Tabacum*, and probably still later in *N. nudicaulis* and its hybrid. This retardation becomes greater for a time, causing the curves to draw farther apart. Later they approach each other again, indicating that the hybrid embryo for a short time near the end of its development grows faster than the pure species embryo. There is always, however, in the crosses studied, a considerable difference in maximum size between the embryos of pure species and those of the corresponding hybrids.

There seems to be no difference between the growth of hybrid embryos in groups III and IV. There is nothing in the growth of group III hybrid embryos upon which to predict ultimate failure.

N. nudicaulis and its hybrid offspring gave less typical curves, due, in the first place, to the fact that the most rapid growth came at a later time. At 21 days when the observations were discontinued both kinds of embryos seemed to be still growing rapidly. Very likely if measurements had been made up to 25 days very much the same type of curve as the others would have resulted.

Besides, in the curve for this hybrid, *N. nudicaulis*×*N. Tabacum*, as well as in the curve for *N. suaveolens*, there are notable irregularities. Each one crosses and recrosses its mate. The *N. suaveolens* embryos, furthermore, according to the data, reached their maximum length at 17 days and then steadily grew smaller—an impossible result, of course. Two possible explanations might be offered for these anomalies. Since the period of making observations extended over fall, winter, and spring, it might be that the embryos which grew during early fall and late spring developed faster than those which grew during the shorter, colder days of winter. Or it might be that sometimes fertilization was effected more quickly. Since age is counted from pollination and not from fertilization this, it might be thought, would give some embryos an uneven start. Possibly the age of the flower when pollinated could be the cause of uneven time of fertilization.

There is some evidence that the former of these factors has some effect. One of the most extreme cases is in *N. nudicaulis*. The average of 14 em-

bryo lengths at 19 days is recorded as 0.1126 mm. The pollinations of these capsules were made on December 31. Seven embryos developing in capsules pollinated on March 28 attained at the same age an average length of 0.4380 mm. In the same species the lengths recorded at 9 days are slightly greater than those for 11 days, very possibly due to the fact that the former group of embryos developed in April, while the latter were produced in December.

On the other hand there are a number of instances where no such effect of the season can be detected. For example, the *N. paniculata* curve is scarcely as regular as those for *N. Tabacum* or *N. suaveolens*×*N. Tabacum;* yet the pollinations for the *N. paniculata* were all made within a nine day period, about May 1, while those for *N. Tabacum* extended from December 12 to May 14, and those for *N. suaveolens*×*N. Tabacum* from October 31 to March 19. Altogether it seems unlikely that the season in which the embryos are produced is a very important factor in determining the size or rate of growth.

Neither is it likely that flower age at the time of pollination has contributed in any important way to the irregularities noted. In the first place, in practically every case the first signs of an embryo were found at 7 days. If some flowers were older when pollinated they were evidently not able on that account to effect fertilization any sooner, and thus to give their embryos a handicap in the race. Further, three *N. rustica* flowers on the same plant but in different stages of development were selfed on the same day to test this point. Unfortunately the capsule from the oldest flower was lost. Five embryos in the capsule from the youngest flower averaged, at 13 days, 0.6482 mm, while three in the capsule from the flower at the middle stage, as ordinarily pollinated, averaged 0.8614 mm, noticeably larger to be sure. Still the difference is probably not significant as even greater variations occurred at times in a single capsule. For example, as an extreme case, the embryo lengths in one capsule ranged from 51 to 122.5 micrometer divisions.

We can only conclude that the irregularities seen in the data are caused by variations due to causes not discovered. There must be other factors at least equally potent in producing these variations with the two already postulated.

Lest the reader be misled it should be noted that by no means all the observations were as erratic as those just cited. If they had been still greater irregularities must have appeared in the curves. A typical set of measurements, in micrometer divisions, for comparison of variability shown, is as follows: 59, 44, 46, 54.5, 58.5, 45.5, 45.5, 58.25, 48.5, 53, 54, 60, 56. These are the measurements of 13 different *N. nudicaulis*×*N. Tabacum* embryos at 21 days.

The causes of the failure of the crosses of groups I and II to grow at all, and of the failure of group III hybrids to continue growth much beyond germination, do not fall within the scope of the present investigation. The only explanations offered are those postulated by KOSTOFF, who suggests immunological relations between the maternal parent and the hybrid embryo which it supports, as one explanation of the incompatibility of species. He also observed a large accumulation of starch outside the nucellus in the ovules containing hybrids between incompatible species, which, he thinks, makes it so difficult for the embryo to get its nourishment that growth can not be carried on.

Certainly there is nothing in the results here presented to support the idea of immunological relations. On the contrary my observations are rather against this view. In the first place several hybrids made no increase in size at all. They merely existed for from 1 to 4 days, making in some cases a number of cell divisions. There seems to be something inherently wrong with the genotype here which does not permit the cells to grow. If one may draw an analogy from serological reactions in animals it would seem that if the embryos fail because of immunological relations, they should make a normal start, the injurious effects of the reaction with the maternal tissues coming only at a later time. Further, this sort of reaction can hardly explain the experience of embryos of the hybrids of group III which apparently complete their development normally, but fail in the seedling stage.

BRIEGER'S (1928) work, like mine, deals with the time of the failure of the hybrid. This writer, however, determines the time in terms of the differentiation of plant tissues, rather than physical size and length of life. His conclusions are summarized as follows: three critical phases occur where the organization of the individual may break down as the result of the incompatibility of the two genotypes: (1) formation of the vegetative growing point and differentiation of vegetative parts in the embryo; (2) differentiation of various floral parts; (3) differentiation of the archesporium or of the haploids from the archesporium.

Brief comment only need be made with reference to the second and third conclusions, which have less to do with the subject in hand, namely embryo development; then we may examine somewhat more fully the findings summarized under number 1. Finally I shall attempt a somewhat more complete analysis of the time of failure of hybrids in Nicotiana than these statements give.

The hybrids which in BRIEGER'S observations failed to develop the floral parts were interspecific Nicotiana hybrids of the F_2 generation and backcrosses. He also quotes HERIBERT-NILSSON (1918) as having observed in F_1 *Salix caprea*×*S. viminalis* the substitution of vegetative leaves for

inflorescences. But in Nicotiana those F_1 hybrids at least which have been able to complete their vegetative growth also produce flowers of the usual type.

BRIEGER'S third conclusion refers to the inability of many hybrids to complete sporogenesis in such a way as to produce functional gametes. This is a phenomenon familiar to botanists generally.

The first part of his summary is correct, I am sure, as regards the fate of certain hybrids, but certainly not all. He pictures a small embryo of the backcross (*N. Tabacum*×*N. Rusbyi*)×*N. Tabacum* var. *Cuba* which is evidently stopped in its growth at a stage comparable to that at which my F_1 hybrids of group II ceased their growth (figure 8). This seems to be, indeed, just before or at the time the growing point, located just between the two cotyledons, appears to be differentiated. But my hybrids of group I, unlike these, were able to undergo very few cell divisions and died surely before any differentiation of cells was initiated. KOSTOFF (1930) made similar observations on his cross *N. paniculata*×*Petunia violacea*, in which case the embryos stopped in the 4–6 cell stage. In still other cases, as in my group III, the embryo completes a seemingly normal development and is still wholly unable to get beyond the seedling stage.

KOSTOFF'S analysis of the situation is more complete than BRIEGER'S. He puts the results of Nicotiana species crosses into five classes.

1. Crosses in which the pollen tubes do not reach the ovary.

2. Crosses in which the pollen tubes reach the ovary, penetrate the micropyle, and induce parthenocarpy.

3. Crosses in which fertilization occurs, but in which the embryos die in a very early stage.

4. Crosses in which the seeds germinate, but the plants die in the seedling stage.

5. Crosses from which mature hybrids are obtained.

As he points out the lines of demarcation are not sharp and there is some overlapping between these categories. It is a helpful device, nevertheless, and from the evidence already presented and the experience of other investigators, three or four other classes could be recognized. KOSTOFF'S group 2 fails to distinguish between my hybrids of groups I and II,—hybrids which make only one or two divisions in the embryo, and those which live and produce a considerable number of cells over a period of about one fourth of the usual period of embryonic development. KOSTOFF himself observed some embryos of this type which developed farther than mine, in some cases to 100 cells, in *N. Tabacum*×*N. Langsdorffii*. There are other cases in which the embryo apparently completes its growth but is unable in most cases to germinate. His classification also omits mention

of the dwarf plants. Doctor E. M. EAST informs me that these form a quite conspicuous group among his Fragaria hybrids, and similar dwarfs have appeared in other genera. These dwarfs, which stop at several rather definite stages of vegetative growth, are still able to bloom. And, lastly, the fact that some vigorous hybrids are fertile while others are sterile justifies making two groups out of KOSTOFF's number 5.

A more complete classification would be:

1. Crosses which produce no stimulation of the ovary with the result that the flower falls. Many such cases were noted in my experience in testing compatibilities of 23 species, as well as in the work of others. KOSTOFF found in his study of crosses of this kind that the pollen tube never entered the ovary, and that it must enter to effect the results given in any of the following groups.

2. Crosses in which the ovary is stimulated but without production of a hybrid embryo.

a. Stimulation stops with inducing some cell divisions and growth in the nucellus (KOSTOFF).

b. Parthenocarpic seeds are produced.

c. Parthenogenetic (maternal) seeds are produced, either haploid or diploid.

3. Crosses in which true hybrids are produced, which die at about the four cell stage, as in my group I and the Nicotiana × Petunia crosses.

4. Crosses in which the true hybrid makes, say, one fourth or more of its normal number of cell divisions, although possibly without any appreciable growtn in size, as in my group II.

5. Crosses which result in completely formed hybrid embryos unable in most cases to germinate. Many crosses resulting in non-viable seeds have been reported (51 in my own tests) but in most of such cases the embryo development was not studied. In some instances, however, 1 to 3 percent of the seed has germinated, indicating that embryo growth was practically completed.

6. Crosses like my group III which make a fair to good germination but always die in the seedling stage. Doctor EAST has unpublished observations which show that in some of these there is an accumulation of starch in the cells of the growing point, which the plant is, for some reason, unable to utilize.

7. Crosses resulting in dwarf plants which remain small but in a sense mature and flower.

8. Crosses which produce vigorous, healthy, sterile plants. My *N. paniculata* × *N. glauca* is a case in point.

9. Crosses which produce fertile, or partially fertile, hybrids.

SUMMARY AND CONCLUSIONS

1. Eight crosses were made, two in each of four different categories based on the success of the hybrid. The resulting hybrid embryos were sectioned and measured at two-day intervals from 7 to 21 days, and growth curves drawn from the data.

2. Fertilization was found to take place usually on the seventh day. There were three exceptions. In *N. rustica* it was earlier as the 7 day embryos had about 32 cells. In *N. Tabacum*×*N. glauca* and *N. rustica* ×*N. Rusbyi* no embryos were found until nine days after pollination.

3. The complete development of the Nicotiana embryo from the fertilized egg is described and illustrated. This development is similar to that found in other dicotyledonous Angiosperms.

4. Two hybrids, *N. rustica*×*N. glauca* and *N. rustica*×*N. longiflora*, of group I, completed only one or two cell divisions and did not grow at all. They lived only a day or two.

5. *N. rustica*×*N. Rusbyi* and *N. rustica*×*N. Palmeri*, representing group II, experienced a number of cell divisions so that 16 to 32 cell stages appeared but failed to make any appreciable growth.

6. With certain irregularities, which are discussed but for which no explanation is found, the curves of all the other crosses are similar and resemble the familiar logistic, S-shaped curve which has been used to depict the growth of many forms of life.

7. The embryos of group III hybrids, which give a partial germination but die soon after, attain their full size as well as those of group IV.

8. No real difference was noted between the growth of embryos of hybrids of group III, *N. nudicaulis*×*N. Tabacum* and *N. suaveolens*×*N. Tabacum*, and that of embryos of hybrids of group IV. In each case the hybrid embryo curve simulated that of the maternal parent but always fell somewhat below it, even in the most successful crosses, *N. Tabacum* ×*N. glauca* and *N. paniculata*×*N. glauca*.

9. Contrary to the findings of BRIEGER who lists only one stage in embryonic development where the hybrid fails, namely, at the time of differentiation of the growing point, I have three groups of hybrids which fail at different stages during embryonic growth or only shortly beyond it.

10. The cause of the failure of hybrids between incompatible species, that is, the nature of the incompatibility, is not studied, but it is pointed out that the observations made lend no support to the hypothesis that it is due to immunological reactions between mother plant and hybrid embryo. The failure of some of the hybrids to make any growth at all suggests rather some inherent physiological inability, dependent upon the unfavorable hybrid genotype.

11. All interspecies crosses are classified in 9 categories, based on the results obtained, ranging from no stimulation in the ovary—complete failure—to the production of fertile or partially fertile hybrids.

ACKNOWLEDGMENT

The writer takes pleasure in acknowledging his indebtedness to Doctor E. M. EAST for plants and other equipment provided, and especially for his help in planning this study and his invaluable guidance and advice throughout the progress of it.

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MUTANTS AND CROSSING OVER IN THE DOT-LIKE CHROMOSOME OF *DROSOPHILA VIRILIS*

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In the oogonial metaphase of *Drosophila virilis*, we find five pairs of the rod-shaped chromosomes similar in length and one pair of the dot-like chromosomes. Hence, in this species, there should be six linkage groups besides the Y chromosome linkage, and one of them is expected to contain fewer genes and to have very low frequency of crossing over.

In the course of genetical investigations with the Japanese stock of *Drosophila virilis*, we have discovered a considerable number of genes which are assigned to six linkage groups (CHINO 1929, 1930 and unpublished data). Of the approximately one hundred genes (the number of genes including multiple allelomorphs in the six linkage groups of *D. virilis:* (X) 35, (II) 19, (III) 11, (IV) 13, (V) 17, (VI) 6) thus far found, the following six are to be assigned to the sixth linkage group in our map.

(1) plain (p_l): (March, 1929, CHINO) Recessive, somewhat yellowish body color; dark streak on thorax indistinct; this stock was lost before other genes were discovered.

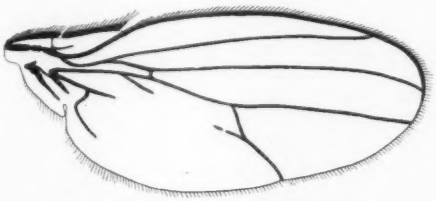

FIGURE 1.—Gap wing of *Drosophila virilis.*

(2) Gap (G_p): (January, 1931, CHINO) Dominant, lethal when homozygous; fifth longitudinal vein almost obliterated between the two crossveins; veins near anterior crossvein somewhat disarranged (figure 1).

(3) stubby (s_t): (April, 1931, CHINO) Recessive; bristles on thorax and scutellum rather shortened, but not attenuated.

(4) stubby-2 (s_{t2}): (April, 1931, CHINO) Recessive; only posterior scutellars shortened and distorted, allelomorph of stubby.

(5) lethal-VIa (l_{VIa}): (February, 1932, CHINO) Discovered in the stubby stock; this makes balanced lethal with the Gap gene.

(6) glossy (g_l): (discovered by American authors) Ommatidia irregular; surface of eyes with oily lustre; eye color reddish. (The material with this

character was originally sent us by Doctor M. DEMEREC. After a linkage test, it has been found that this character is to be assigned to the VI chromosome in our map, though it had been located on the IV chromosome in the American map by METZ, MOSES and MASON [1923].)

Besides these six genes, METZ (1923) and DEMEREC (unpublished data) have reported several genes which belong to their sixth chromosome, namely Net, Minute, bent and Pale. But, as the results given later indicate, the genes which are assigned to the sixth linkage group in our map undoubtedly belong to the small chromosome, so that it seems to be desirable to change the original nomenclature published by METZ, etc., and call their IV linkage group the VI linkage group, if it is proper to name the small chromosome the VI chromosome, as METZ and his associates seem to consider (METZ, MOSES and MASON 1923, pp. 77–78).

CROSSING OVER EXPERIMENTS

Owing to the dominant character of the Gap gene and the phenotypic clearness with which all the genes express themselves, crossing over experiments on this chromosome can be readily performed. The result of our first experiment to combine Gap with glossy is given in table 1.

TABLE 1

Progeny of Gap/glossy×glossy (July 1931).

NUMBER OF CULTURE	GAP	GLOSSY	GAP, GLOSSY	+	TOTAL
1941	146	145	2	4	297
1942	140	144	4	3	291
1943	120	149	0	0	269
1944	157	163	2	0	322
1945	147	151	1	2	301
1946	139	161	0	1	301
Total	849	913	9	10	1781

Crossing over, 1.06 percent

As this table shows, the two parental combinations in the non-crossover class are approximately equal as are the two new combinations in the crossover class. The frequency of crossing over between these two genes is only 1.1 percent. From these facts, we may infer that crossing over has actually occurred in this chromosome, even though the frequency is very small.

From culture No. 1942 we were able to make one stock combining Gap and glossy in the same chromosome. The result of the unsuccessful second experiment to combine this stock with stubby is given in table 2.

Here, if the frequency of crossing over were about 1.1 percent as pre-

TABLE 2

Progeny of Gap, glossy/stubby×glossy (October 1931).

NUMBER OF CULTURE	GAP, GLOSSY	+	GLOSSY	GAP	TOTAL
2002	134	142	0	0	276
2003	124	133	0	0	257
2004	153	128	0	0	281
Total	411	403	0	0	814

Crossing over, 0.0 percent

viously observed, about eight crossovers would be expected among the total of 814 flies. As the cause of the discrepancy between the results of these two experiments the following possibilities may be considered: (a) the genic difference between the stocks used in these experiments, (the frequency of crossing over varies in *D. virilis* according to the strain used); (b) the possible difference in the frequency of crossing over between the cases of coupling and repulsion; and (c) the variation due to external influence, such as temperature, since the first experiment (table 1) was carried out during the summer, while the second (table 2) was performed during the autumn.

In order to solve the above question, we performed the following experiment. Larvae having s_t, G_p/g_l constitution derived from the same

TABLE 3

Progeny of Gap, stubby/glossy×glossy, at 30° C and 25° C.

TEMPERATURE	GAP	GLOSSY	GAP, GLOSSY	+	TOTAL
	95	82	0	0	177
	67	81	0	0	148
30° C	47	74	1	0	122
	49	55	0	0	104
	64	75	1	0	140
	82	76	1	0	159
Total	404	443	3	0	850
	82	90	0	0	172
	76	105	0	0	181
	94	92	0	0	186
25° C	91	100	0	0	191
	68	67	0	0	135
	83	109	0	0	192
Total	494	563	0	0	1057

Crossing over at 30° C, 0.34 percent
Crossing over at 25° C, 0.00 percent

parent were divided into two groups and they were bred at 30°C and 25°C respectively. After their emergence, only the females showing Gap character were selected and mated to glossy males, and were bred, as before, at 30°C and 25°C respectively. The results are given in table 3.

As this table shows, each of the three cultures among the six at 30°C gave rise to one crossover individual, whereas no such crossover was found among the six cultures at 25°C, in spite of the greater total number of flies than in the former case. It is, therefore, very likely that high temperature raises the frequency of crossing over between these two genes, even if we admit the other two possibilities, especially the one mentioned under (a). Further details on these points are left for a future study.

The same relation seems to hold also for the region between Gap and stubby, although we have not yet made any detailed study of this matter. Several experiments completed thus far have given the following result in the case of $G_p/s_t \times s_t$ matings: Gap, 1228; stubby, 952; Gap, stubby, 3; wild, 6. The frequency of crossing over here is 0.41 percent.

The locus of lethal-VIa has not been found as yet, but, judging from the fact that it makes a balanced lethal with Gap, it can be assumed that the gene is located near the three genes described before.

Thus both the data concerning the number of mutants and that concerning the frequency of crossing over suggest that those genes belong to the small chromosome in this species and form the sixth linkage group. This conclusion is strongly supported also by the fact that the crossing over in our IV linkage group is high. For instance, the percentage of recombination between plexus and dachsous is 46.3, between plexus and irregular is 53.5, and that between dachsous and irregular is 22.1 (CHINO 1929, 1930 and unpublished data). This proves that the IV linkage group in our map belongs to one of the longer chromosomes.

HAPLO VI IN *Drosophila virilis*

KIKKAWA, one of the authors, found a female (December 1931) with glossy eyes among the F_1 progeny of glossy×wild mating. This fly was about $\frac{2}{3}$ of the normal fly in body size, and had small, slender bristles, blunt and slightly spread wings, and looked weaker than normal. This fly represents probably haplo VI or diminished in *D. virilis*, or the deficiency at the glossy locus. Unfortunately it was sterile.

DISCUSSION

In the fourth linkage group in *D. melanogaster*, the following six characters have hitherto been reported: bent, shaven, eyeless, Minute IV, dominant eyeless and rotated abdomen (MORGAN, BRIDGES, STURTEVANT 1925, 1926, MULLER 1930, PATTERSON and MULLER 1930, BELIAJEFF

1931). But, in *D. virilis*, no similar mutant has been found except bent previously reported by METZ, etc. (Very recently CHINO discovered a mutant, rotated abdomen, which seems to correspond to the similar mutant in the IV chromosome of *D. melanogaster*. After a linkage test, it was found that the mutant belongs to the VI chromosome in our map. However, no certain case of crossing over between it and Gap has been found as yet).

Lately, BOLEN (1931) found in the study of X-IV translocations in *D. melanogaster* that the eyeless gene is located at a point distal of the bent gene. However, before this discovery of BOLEN, it has been assumed that the orders of these genes can not be determined from crosses since the crossing over does not occur in this chromosome, and that the cases of apparent crossovers previously reported were probably due to non-disjunction or phenotypic overlapping of genotypic classes (MORGAN, STURTEVANT, BRIDGES 1926, PATTERSON and MULLER 1930).

But, in the dot-like chromosome of *D. virilis*, as our experimental result has proved, crossing over does actually occur, although there is suspicion that some of the cases given before are nothing but the result of non-disjunction. If the glossy and the stubby genes can be put into the same chromosome, we shall be able to decide the orders of arrangement and also the relative distance of these two recessive genes from the Gap gene by a crossing over test. Such an attempt is now underway.

The discrepancy noted between the two species of Drosophila concerning the crossing over in the dot-like chromosome may be accounted for to some extent as follows. The X chromosomes seen under the microscope do not show much difference, either in shape or length, between the two species; the genetical maps of this chromosome, however, show a rather considerable difference, comprising about 182 units in *D. virilis* (CHINO 1929), but only 66 units in *D. melanogaster*, (MORGAN, BRIDGES and SCHULTZ 1931, the ratio being 2.8 to 1.

A similar relation is found between the autosomes in the two species. Thus, it may be assumed that crossing over on the whole occurs more frequently in *D. virilis* than in *D. melanogaster*. If so, it is not very surprising if a few cases of crossing over should be found in the dot-like chromosome of the former species.

This fact seems to justify the view that every species has an *a priori* frequency value of crossing over as a whole which is independent of the length of the chromosomes. As a matter of fact, TANAKA (1925) has reported a case in the silkworm where the frequency of recombination exceeds 45 percent notwithstanding the oval shape of all the chromosomes, and HUXLEY (1923) advances a similar view in his work on *Gammarus chevreuxi* where the chromosomes have similar shape.

SUMMARY

1. Six factors, located in the dot-like chromosome of *D. virilis*, are described: plain, Gap, stubby, stubby-2, lethal-VIa and glossy.

2. Crossing over was found to occur between Gap and glossy, and also between Gap and stubby, the frequency being about 0–1 percent in both cases. The frequency seems to be increased by high temperature.

3. One female probably lacking one dot-like chromosome (haplo VI) was described.

4. A plausible explanation is given for the question as to why the crossing over is found in the dot-like chromosome of *D. virilis*, while it is not found in the similar chromosome of *D. melanogaster*.

ACKNOWLEDGMENT

In conclusion the authors wish to express their deep obligations to Professor Doctor TAKU KOMAI for his valuable advice and the trouble he took to look through this manuscript. To Doctor M. DEMEREC, also, we are indebted for valuable criticism.

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CUBITUS INTERRUPTUS, A NEW GENOVARIATION OF THE FOURTH CHROMOSOME OF *DROSOPHILA MELANOGASTER*

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The new genovariation here described was discovered in the Normal Florida line in February, 1930, simultaneously at the INSTITUTE OF EXPERIMENTAL BIOLOGY in Moscow and at the CENTRAL GENETICS STATION of Narcomzem (Department of Agriculture). At the CENTRAL GENETICS STATION, the gene-variation was named cubitus interruptus (c_i) and it was called cubitus incompletus in the genetics laboratory of the INSTITUTE OF EXPERIMENTAL BIOLOGY.[1]

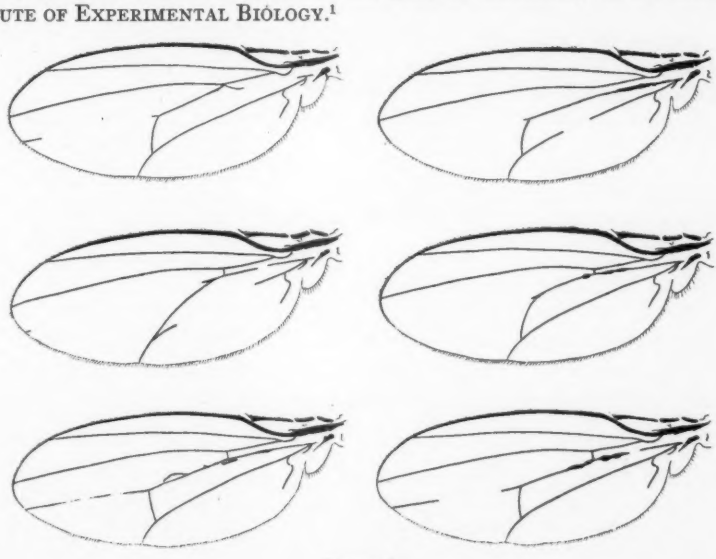

FIGURE 1.

PHENOTYPE OF THE GENOVARIATION

The mutant character consists of one or of several interruptions of the fourth-longitudinal vein (cubitus). Sometimes, additional interruptions are observed in the fifth-longitudinal vein and in the posterior cross vein, which, at times, is even entirely lacking. Sometimes also, the cubitus and

[1] The authors are indebted to Pros. H. K. Koltzoff, Director of the Institute of Experimental Biology for numerous helpful suggestions.

the second cross vein are crooked, and frequently the cubitus is thickened and ragged at the basis. The drawings of six wings in figure 1 give a clearer demonstration of the comparatively strong invariability in the expression of this character. The manifestation of the character in the lines obtained at the CENTRAL GENETICS STATION was complete (100 percent). In the lines of the INSTITUTE OF EXPERIMENTAL BIOLOGY there were inbred cultures where the average manifestation of the character was 98 percent. It is necessary to note that in some lines the percentage of the character manifestation was considerably lower, this being evidently due to the difference of the genotypical medium. The character is very often asymmetrical in its expression, and is sometimes lacking on one of the wings.

GENETIC ANALYSIS

In F_1 from crosses of the new mutation with normal lines (Florida and Caucasus Gelendzhik) exclusively normal individuals, in males as well as in females, were obtained (see table 1). Hence it follows that the new genovariation is recessive and not sex-linked. In F_2 a ratio very near to 3:1 was obtained (see table 1).

TABLE 1

CROSSES	F_1	F_2
♀ c_i × ♂ Normal Florida	1,302 Normal	2,317 Normal: 641 c_i
♀ Normal Florida × ♂ c_i	365 Normal	(3.13:0.87)
♀ c_i × ♂ Caucasus Normal	353 Normal	2,557 Normal: 786 c_i
♀ Normal Caucasus × ♂ c_i	185 Normal	(3.06:0.94)
Total	2,205 Normal	4,874 Normal: 1,427 c_i (3.09:0.91)

For determining in which of the autosomes the c_i gene is situated, the following dominant mutations were used as indicators: for the second chromosome, Lobe 2 (L^2) and Star (S), and for the third, Dichaete (D). As a result of the backcrosses of the F_1 heterozygotes with the double recessives, the data presented on tables 2 and 3 were obtained. In these data the relative lowness of the numbers of c_i and the relative highness of the corresponding not-c_i class (for example, 556 > 363) is probably due to strong overlapping of the c_i character with the not-c_i phenotype. This

TABLE 2

Male backcrosses for chromosome II.

c_i	L^2	c_i L^2	NORMAL
363	595	387	556
c_i	S	$c_i S$	Normal
99	203	157	217

TABLE 3
Male backcrosses for chromosome III.

c_i	D	$c_i D$	NORMAL
337	307	296	505

suggests that the stocks in which the expression is 100 percent or 98 percent have modifying genes which strengthen the manifestation.

It is seen from these tables that the flies obtained in the male backcrosses were of 4 phenotypes, which shows that no linkage exists either with the second or with the third chromosome characters. One supposition remained possible, that of the gene c_i being linked with fourth-chromosome characters. To verify this hypothesis a cross was made between homozygous c_i, flies and homozygous flies of the fourth-chromosome character eyeless (Russian mutant allelomorph). All the flies obtained from this cross (527 in number) were normal, as was to be expected, both the genes being recessive, and the flies of this generation being heterozygous by both genes. In the second generation, a segregation into 3 phenotypes, in the proportion of 2 Normal: 1 $e_y{}^R$:1 c_i was expected, and was realized, as table 4 shows.

TABLE 4
F_2 from the cross of $c_i \times e_y$.

NORMAL	c_i	$e_y{}^R$	$e_y{}^R\ c_i$
3,220	1,422	1,008	0

If the gene c_i were not carried in the fourth chromosome, we ought to have received four phenotypes in the ratio of 9 Normal: 3 c_i; 3 $e_y{}^R$:1 $e_y{}^R c_i$. Since no phaenotype $e_y{}^R\ c_i$ was obtained the data prove that the c_i gene is carried in the fourth chromosome. One of the authors of the present paper (G. G. TINIAKOF) has obtained through crossing over in the fourth chromosome between eyeless (Russian) and c_i, a stock of $e_y{}^R\ c_i$.

We had the opportunity of demonstrating this stock to BRIDGES on his visit to our Institute in January, 1932, and he confirmed our classification. He also stated that this is the first definite evidence of crossing over in the fourth chromosome. His own experiments had given apparent crossing over (0.9 between eyeless and bent), but some of this apparent crossing over was undoubtedly due to non-disjunction instead and he had not excluded the possibility that all of it was due to non-disjunction and that perhaps the fourth chromosome was too short to give the usual kind of crossing over. With the $e_y{}^R\ c_i$ stock further work is now being carried on for testing the amount of crossing over between the genes in question. The results will be published later.

SUMMARY

The new character here described is due to a recessive gene in the fourth chromosome in *Drosophila melanogaster*. The gene causes varying breaks and abnormalities in the veins of the wing, principally in the cubitus and occasionally in the analis and in the posterior cross vein. In some lines the genovariation c_i has a complete manifestation (100 percent) while in other lines it is 98 percent or lower; in the F_2 from out-crosses it may be as low as 75 percent. This probably means that the high manifestation stocks have intensifying modifiers which are separated from c_i in the segregation. Definite evidence that crossing over occurs in the fourth chromosome between the loci eyeless (Russian) and c_i was obtained.

CHIASMA FORMATION IN LARIX AND TSUGA*

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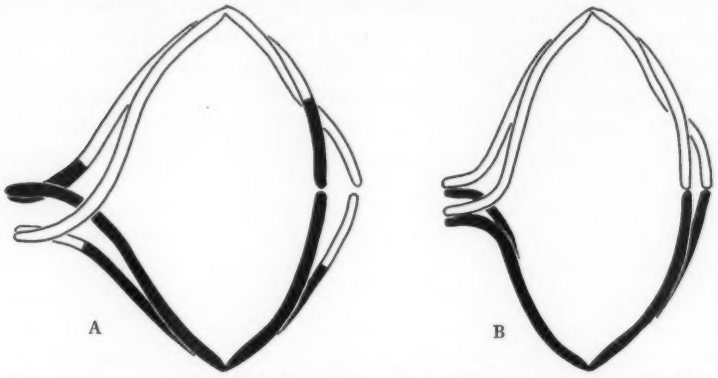
From the standpoint of chiasma formation and the relation of the chromatids, two essentially different theories have been advanced. The first is JANSSENS' "partial chiasmatypy" hypothesis (1924). This theory enjoins a crossing over between two of the four chromatids, a crossover at every chiasmata, and a pairing of only sister threads at early diplotene. The Janssens' "partial chiasmatypy" theory, with various modifications, is supported by BELLING (1931), DARLINGTON (1931), and MAEDA (1930).

The other hypothesis is that of MCCLUNG (1927). The work of WENRICH (1916), ROBERTSON (1916), CAROTHERS (1926, 1931), NEWTON (1927), BELAR (1928), and many others supports this theory. Chiasmata are produced by an alternate pairing of sister and non-sister strands. Chiasmata represent an exchange of partners among the chromatids. According to SAX (1930), a crossover occurs only when two chromatids break at a chiasma. On this theory chiasmata may be present without crossing over.

MCCLUNG (1927) has pointed out clearly that when there is crossing over, according to JANSSENS' theory, there should be an asymmetrical arrangement of the chromatids. JANSSENS based his arguments for his theory on cases where the asymmetrical arrangements have been found in certain Orthoptera, but as MCCLUNG has pointed out the clearest figures show that symmetrical figures are the usual and most prevalent types. On any theory, crossing over between two of the four chromatids should usually result in an asymmetrical relation of the chromatids. If a single crossover occurs it is possible that the chromatids might be subsequently oriented to produce a symmetrical relation of the chromatids, but in case two crossovers occur in a single bivalent a symmetrical relation of chromatids should be exceptional. Viewed from the end, each of the four chromatids should not lie in the same quadrant at all loci after a crossover has occurred at the four strand stage. As the homologues open out, the chromatids which were adjacent may form the cross at the chiasma if the chiasma is the result of a reciprocal crossover (text figure 1A). As such a chiasma is terminalized there should be a twist in one pair of chromatids. These reciprocal crossovers should occur as frequently as equationals if crossing over is at random. Equational crossovers should also result in asymmetrical relations of the chromatids although it is possible that chro-

* The cost of accompanying heliotype plate is paid by the GALTON AND MENDEL MEMORIAL FUND.

matid movements in subsequent stages might produce symmetrical arrangements of chromatids in some cases. If each chiasma represents a crossover as is postulated in JANSSENS' "partial chiasmatypy" hypothesis, the bivalent chromosomes with one or more chiasmata should show a prevalence of asymmetrical chromatid relationships.



TEXT FIGURE 1.—A. Chromatids at metaphase stage of meiosis according to the Janssens hypothesis. The arrangement of the chromatids is asymmetrical. The chiasmata represent crossing over. The chiasma at the left is formed by adjacent chromatids. On the right the chiasma has terminalized. There is a twist in the upper pair of chromatids. B. Chiasma formation according to the McClung theory. The cross in the chiasma at the left is formed by diagonal chromatids. At the right the chiasma has terminalized and the asymmetrical arrangement is due to a half twist at early prophase.

On MCCLUNG'S theory, chiasma formation is not the result of previous crossovers, so that a symmetrical arrangement of chromatids might be expected if there are no crossovers or no twists in sister chromatids. Each chromatid should lie in the same quadrant at all loci at the four strand stage, and as the paired chromatids open out at diplotene and metaphase the apparent cross at the chiasma is formed by chromatids which were diagonal at the four strand stage (text figure 1B). A half twist in sister chromatids before or after pairing of homologous chromosomes would produce an asymmetrical relation of chromatids even if no crossover has occurred (text figure 1B). A crossover resulting from a break in two chromatids at a chiasma (SAX 1932) would result in an asymmetrical relation of chromatids.

A prevalence of symmetrical chiasmata have been shown in Orthopteran chromosomes by WENRICH (1916), ROBERTSON (1916) CAROTHERS (1927) and DARLINGTON (1932). As MCCLUNG (1927) has pointed out, JANSSENS' (1924) clearest figures also show a symmetrical relation of chromatids. Both symmetrical and asymmetrical chiasmata have been found in Paeonia (SAX 1932b). The symmetrical double chiasmata show clearly

that no crossovers have occurred in these chromosomes and that chiasma formation is not dependent on a previous crossover. Asymmetrical chiasmata would be expected if a crossover has occurred at another chiasma or if a half twist was formed between sister chromatids. In some cases the cross at the chiasma was formed by the two threads which were adjacent at the four strand stage. If, as the work of WENRICH (1916) and CAROTHERS (1931) suggests, sister chromatids are not always associated at the fiber attachment point, these chiasmata might be attributed to an equational separation at the first meiotic division associated with a half twist in sister chromatids.

MAEDA (1930) has observed asymmetrical chiasma formation in *Lathyrus odoratus*. This would be expected as crossing over occurs in *Lathyrus odoratus*, and on any theory of crossing over asymmetrical chiasmata are formed in crossing over.

CHIASMATA AND CHROMATIDS IN LARIX AND TSUGA

The present investigation is concerned with chiasma formation and the relation of the chromatids in Tsuga and Larix. *Tsuga canadensis*, *Larix decidua*, *Larix Kaempferi*, and the hybrid *Larix eurolepis* Henry (*L. Kaempferi*×*L. decidua*) offer very good material for the study of the chromatids.

Permanent smears and paraffin sections of the microspore mother cells of Larix and Tsuga were made. The material was fixed in Navaschin's, La Cours', and Darlington's modification of Flemming's stronger fixatives. Permanent smears with the latter fixative gave the best results. Crystal violet iodine gave the best results in staining the chromosomes. Flemming's triple stain was a better stain for the fibers.

The divisions in the microspore mother cells of *Tsuga canadensis* are especially favorable for the study of chiasmata. The term "chiasma" will be used here to designate the region where there is an exchange of partners among the chromatids. The chiasmata are very similar to those found in Larix (SAX 1932, plate 51), although the chromosomes are somewhat larger. During diakinesis the configurations of chromosomes and the number and position of chiasmata can be observed. The prevalence of interstitial chiasmata suggests that there is very little terminalization before the metaphase stage.

Figure 1, plate 1, shows all the chromosomes drawn from a side view of a late metaphase stage in the first division of a microspore mother cell of *Tsuga canadensis*. They are drawn separately to show the detail of each one. The number and position of the chiasmata can be clearly seen in some of the chromosomes, while in others some of the chiasmata have opened out because of the lateness of the stage. This point is easily inferred

LEGEND FOR PLATE 1

All drawings were made from the first division of the microspore mother cells, with the aid of a camera lucida. The magnification is ×3100.

FIGURE 1.—Twelve chromosomes, a, b, c, d, e, f, g, h, i, j, k, and l are drawn from a single late metaphase stage in the first division of the microspore mother cell of *Tsuga canadensis*. The number and type of chiasmata are shown.

FIGURES 2 and 3.—Bivalents of *Tsuga canadensis*.

FIGURE 4.—Bivalent from *Larix eurolepis* at diakinesis stage with two interstitial chiasmata.

FIGURE 5.—Chromosome from *L. eurolepis* in late diakinesis. Two chiasmata, one subterminal, the other interstitial are present. The chromatids are separated in the homologues.

FIGURE 6.—Chromosome—late metaphase in *L. eurolepis*. Chromatids symmetrical.

FIGURE 7.—Chromosome at late metaphase in *L. eurolepis*. Chromosome shows stretching between chiasma and fiber attachment.

FIGURE 8.—Bivalent, late metaphase in *L. eurolepis*. One interstitial chiasma. Symmetrical arrangement of chromatids.

FIGURE 9.—Bivalent, late metaphase in *L. Kæmpferi*. Two subterminal chiasmata.

FIGURES 10 and 11.—Metaphases of bivalents of *L. eurolepis* showing symmetrical arrangement of chromatids widely separated with two interstitial chiasmata.

FIGURE 12.—Metaphase of chromosome of *L. eurolepis* with chromatids separated on left side. Symmetrical arrangement of chromatids.

FIGURES 13 and 14.—Chromosomes with fiber attachment, terminal or subterminal. Chromatids separated. Chromatids symmetrical.

FIGURE 15.—Chromosome, *L. eurolepis*, early anaphase. Asymmetrical arrangement of chromatids.

FIGURE 16.—Chromosome in late metaphase, *L. eurolepis*. One chromatid from each homologue separated, the remaining chromatids still paired. Symmetrical arrangement of chromatids.

FIGURE 17.—Anaphase stage of bivalent, *L. eurolepis*. Asymmetrical arrangement.

FIGURE 18.—The same, showing symmetrical arrangement.

a
b
c
d
e
f
g
h
i
j
k
l
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

from the large number of stages at diakinesis and metaphase where the chromosomes have not started to divide. There are several chromosomes (figure 1a, d, h, i and k), where the homologues have opened out at one end. In figure 1l, the homologues have completely separated. There are interstitial and subterminal chiasmata which are very distinct in most of the chromosomes shown in figure 1. Some of the chromosomes show an opening where the chromatids change partners. There is considerable strain on the homologues between the chiasma and the point of fiber attachment. They are very narrow in this region. This is shown in Tsuga (figure 1a, d, i, and k) and in Larix (figure 7).

With very good fixation and lighter stain only the chromatids are seen. The relations of the chromatids at the chiasmata are somewhat more distinct. Figures 2 and 3 from Tsuga show a twisting of the free ends of the chromatids near the chiasmata.

The chromosomes in the divisions of the microspore mother cells of Larix were especially favorable for the study of the chiasmata and the relations of the chromatids in the bivalents. In the hybrid *Larix eurolepis* the pairing between the chromatids appears to be a little less close, and, in some cases, the individual chromatids are distinctly separated. Though perhaps not so distinct, the chromatids are found to have a similar arrangement in the parents, *L. Kaempferi* and *L. decidua*. In most cases, however, in both parents and hybrids the pairing was too close to follow the chromatids throughout, but the chiasmata are quite distinct and the chromosomes very similar in the hybrids and parents. However, a large number of diakinesis, metaphase, and anaphase figures were found in the hybrid in which the chromatids were distinct, and from these most of the drawings were made.

Figure 4 is drawn from a late diakinesis stage. There are two interstitial chiasmata. The homologues are widely separated between the chiasmata and at the free ends beyond the chiasmata. The chromatids are not separated in this case.

Figure 5 represents a late diakinesis, or very early metaphase stage. The chromatids in the two homologues of the bivalent are separated from each other so that they may be clearly seen. There may be a twisting in the chromatids in this case in the lower half. One chiasma is median between the fiber attachment and the end of the chromosome; the other is subterminal. The homologues are again widely separated except at the chiasmata.

In figure 7, a late metaphase, there is an interstitial chiasma with a distinct opening where the chromatids exchange partners, a slight coiling of the free ends of the chromosomes beyond the chiasma, and a stretching of the chromosome between the chiasma and the fiber attachment, indicating some strain in the separation of the paired chromatids.

In figure 6, which is a very late metaphase, the chiasma is subterminal. The paired chromatids are distinct and are separating at the chiasma. The arrangement of the chromatids is symmetrical.

The details in the separation of the paired chromatids in an interstitial chiasma are easily seen in figure 8. If one imagines the uppermost pair of chromatids rotated from the plane of the plate through the vertical position and to a position making an angle of 180° with the present position, one will recognize that the arrangement of the chromatids in this case is similar to that in figure 6; but the chiasma is interstitial. The arrangement of the chromatids in figure 8 is obviously symmetrical. The diagonal chromatids form the cross. In other words, the union of the homologues here is face to face, and not at an angle and lateral.

Two chiasmata, both subterminal, with a comparatively wide space between the chromatids, are seen in figure 9, which was drawn from *L. Kaempferi*. The arms of the bivalent between the fiber attachment and the chiasma show some coiling.

In figure 10, the chromatids are widely separated so that, except at the point of fiber attachment, they are very distinct and easy to follow. The chiasmata are interstitial. The chromatids from the upper homologue pair separately with those from the lower homologue. Two of the pairs of chromatids are touching at the ends. The other two are paired, but they are not in contact in any place. The chromatids are symmetrical on both sides. No asymmetrical arrangement is possible with such wide separation of the chromatids. If the cross had been made by adjacent chromatids, they would not be free to separate so widely in interstitial chiasmata.

Figure 11 is similar to figure 10, with somewhat thicker chromatids. The chromatids to the left have a connection near the distal end, which may be due to a twisting about each other. It is obvious, however, that the arrangement of the chromatids is symmetrical, since the chromatids of the homologues are separated beyond the region of the chiasmata. On the left side of the bivalent shown in figure 12, the chromatids are symmetrical. They are separated throughout their length.

In figures 13 and 14, the symmetrical arrangement of the chromatids is apparent. The spindle-fiber attachment is near the end of the chromosome. The paired chromatids are separated, and the ends of the homologues are separating.

In figure 15, the chromatids are all distinct, and the ends, where the homologues have paired, are separating. There is an appearance of an asymmetrical arrangement in this case.

A slightly more advanced stage is found in figure 16. The symmetrical arrangement of the chromatids is the only one possible in this case. The fiber attachment is in the middle of the homologues of a bivalent with two chiasmata. The chromatids are very distinct. One chromatid on each side

has separated completely at the region of pairing with its homologue, and has its free end in the position characteristic of the anaphase. The other two chromatids of the homologues were still paired at the ends. The arrangement of the chromatids is obviously symmetrical. An asymmetrical arrangement of the chromatids is impossible in this case.

The arrangement is asymmetrical in figure 17. In this case there is a distinct twist in the chromatids in the upper half of the figure.

Figure 18 is an anaphase. The configuration of the chromatids appears to be symmetrical. The chromatids are distinct. They are held together only at the spindle fiber attachment. In the lower half of the figure, they are loosely associated at the region of the spindle fiber attachment point.

DISCUSSION

The figures described above show a great preponderance of symmetrical figures. Though a thorough search was made among the figures of Larix showing separate chromatids, to find chromosomes where the arrangement of the chromatids was obviously asymmetrical, only the few possible cases mentioned above were found. There was manifest almost a total uniformity of symmetrical configurations in the chromatids of Larix.

The greater frequency in the occurrence of symmetrical configurations in Larix supports the McClung theory. They could not occur if the chromatids had paired according to the Janssens hypothesis.

Very few cases were found where an asymmetrical arrangement of chromatids is found in Larix. It is possible in some figures, where a symmetrical arrangement is found, that an asymmetrical arrangement existed earlier and that through a readjustment in the chromatids this asymmetry was straightened out. This may occur where the spindle fiber attachment is near the end of the chromosome. Figures 13 and 14 represent chromosomes of this type. The configurations are obviously symmetrical.

However, a large number of bivalent chromosomes with two chiasmata were found where there was no possibility of an asymmetrical arrangement of the chromatids, which would be expected on the Janssens hypothesis. This arrangement is in agreement with the McClung theory. According to SAX, some cases of asymmetry would be expected on the McClung theory if there was a half twist in sister chromatids before pairing in the early prophase, or if crossing over had taken place.

SAX (1932) found some cases in Paeonia where adjacent chromatids form the cross at the chiasma. This may seem to support JANSSENS' "partial chiasmatypy" theory. These cases may be explained on the McClung theory if non-sister threads are held together at the fiber attachment associated with a half twist in one pair of sister chromatids. From the behavior of heteromorphic chromosomes in Orthoptera, according to WENRICH and CAROTHERS, an equational first meiotic division seems to be possible.

The preponderance of the symmetrical arrangement of the chromatids in Larix suggests that there is little crossing over in Larix, even though the chiasma frequency is relatively high. Previous investigations have shown that the rarity of polyploidy in the conifers may be attributed in part at least to the high chiasma frequency (SAX 1932). The symmetrical chiasmata indicate that little or no crossing over occurs in this group of plants. These two factors, uniformity in chromosome numbers and absence of crossing over between chromosomes, may be of importance in explaining the relative stability of the conifers. In the genus Larix, distinct species which have been geographically isolated for long periods of time produce fertile hybrids, and in general the genera of conifers are not highly polymorphic.

SUMMARY

The divisions in the microspore mother cells of Larix and Tsuga are especially favorable for the study of the arrangement of chromatids because the chromatids are often seen separated in the homologues. In most cases the chromatids are symmetrical in arrangement, and the cross in the exchange of partners is formed by diagonal chromatids. These configurations seem to be incompatible with the present interpretations of the Janssens hypothesis. In a very few cases there are asymmetrical configurations. This arrangement of chromatids would be expected on the McClung theory if either a half twist of sister chromatids or a crossover had occurred in the prophases. The preponderance of symmetrical figures, together with little terminalization of the chiasmata up to metaphase, would indicate that little crossing over occurs and that chiasma formation may be independent of crossing over.

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THE INHERITANCE OF ALLERGY WITH SPECIAL REFERENCE TO MIGRAINE[1]

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For some years during the course of treatment of different forms of allergy at the BALYEAT HAY FEVER AND ASTHMA CLINIC in Oklahoma City, various facts concerning the inheritance of allergy have been noted. It is the object of the present study to determine as far as possible from the material thus collected the type of inheritance displayed by that form of allergy called migraine. The study was made entirely from a genetic point of view and the material used began with the available records of patients of the BALYEAT HAY FEVER AND ASTHMA CLINIC.

Studies on the heredity of allergy are not numerous. Work seems to have begun about 1909 with DRINKWATER'S results which indicate that asthma acts as a dominant character. Since that time a number of investigators have written on some phase of allergy. Some, though reporting on one form of allergy, have realized that various others have occurred in one family. A connection of migraine with the sex endocrines has been observed, several investigators noting that affected females suffer with migraine more frequently during the menstrual period.

ADKINSON has made an extensive study of bronchial asthma (1920) and she concluded that it is a recessive character, since normal people transmit the asthmatic tendency. These normals she regarded as heterozygous. Her findings do not all bear out this conclusion, for she states that children of two heterozygous normals are three times as likely to be asthmatic as normal, whereas one fourth should be asthmatic if the character is a true recessive. She seems to have overlooked the possibility of incomplete dominance. ADKINSON found that bronchial asthma, hay fever, some urticaria, and eczema must be accepted as equivalent in their inheritance. She states that "asthma is inherited with equal frequency from the family of either parent."

BUCHANAN in 1920 considered migraine as a recessive trait. SMITH (1922) concluded that migraine is a dominant trait, in some way sex determined. The work of STUDY, referred to by BAUR, FISCHER, LENZ, indicated that hay fever is a dominant character, manifesting itself in the older members of a family as gout, but in the younger as hay fever. GÄNSSLEN (1921)

[1] Studies from the Zoological Laboratory of the University of Oklahoma, Second Series No. 114.

showed that one individual, and sometimes a number in the same family, often are subject to hay fever, asthma, urticaria, and eczema, gout and gall stones.

LENZ (in BAUR, FISCHER, LENZ), using the charts of UNGER and WIETZ, concluded that migraine is probably a sex-linked dominant and that "the sexual life seems to have an influence upon the occurrence of migraine. There are women who suffer from migraine only when they are menstruating, and never have an attack during pregnancy."

FETSCHER (1927) suggested that hay fever is sex-linked. He found that sons only were affected if both parents were negative and daughters were affected if one parent was allergic. Since he did not prove that hay fever is transmitted to sons from their mother only, and since he showed only three charts, his results are not conclusive.

ALLEN (1927–28) analyzed 400 cases of migraine and concluded that it is inherited as a dominant character. He found that menstruation influenced the onset of migraine. JORDAN (1928) published a family record to show that eczema is inherited.

In 1928, BALYEAT showed that inheritance appears to be the chief factor in determining hay fever and asthma and that the earlier in life an individual becomes sensitive, the greater the tendency to develop a sensitivity to more than one group of proteins. He found that the environment to which an individual is exposed has much to do with the sensitivity which develops. Also he stated that eczema and migraine are interchangeable with hay fever and asthma, and that the thing inherited is the ability to become sensitive, but not the specific state, and that this is inherited as a dominant character.

In 1929, the writers of this paper made a preliminary genetic study of twelve family pedigrees. They concluded that there is probably only one gene involved in the different allergies and that the gene acts as a dominant Mendelian unit, although its dominance is often incomplete, that it sometimes skips a generation, and that the environment is at least to some extent responsible for the expression of this gene.

In 1930, APPERLY published a family tree showing five generations, in each of which allergy occurred. In the same year ELY showed that migraine is an hereditary disease.

In the study of the inheritance of migraine from a genetic point of view it might be of interest to point out some clinical observations. BALYEAT (1931) in a study of a series of 202 cases of migraine concluded that migraine appears to be common in children since nearly one-third of all cases developed symptoms during the first decade, that the syndrome is more common in business men, professional men and teachers than in laborers, that about 7 percent of all people in the United States some-

time in life suffer from the symptom complex, that not unlike asthma symptoms of migraine in childhood may vary greatly from those of the adult, and that migraine is interchangeable in the linkage with other allergic diseases. In an unpublished paper (RINKEL and BALYEAT 1932) in a series of 65 cases of headache (migraine) proved to be due to food hypersensitiveness, a family history of headache was obtained in 84.4 percent. It appears from our clinical study that a patient transmits from one generation to another only the ability to become sensitive and not the specific state. Apparently the type of sensitivity from which the antecedent suffered has no relation to the type the descendant may have.

In using the term "migraine," we refer to those patients who have paroxysmal headaches characterized by hemicrania (also bilateral headaches), and who have symptomatic evidence of cortical involvement. There are headaches due to hypersensitiveness that cannot be classified as migraine since they do not have cortical features. These cases have not been used in our study. It may be remarked that this restricted definition of migraine makes even more striking some of the genetic situations later described, in which persons who have only migraine transmit several other allergies to their descendants.

The methods used in the present work are similar to those by which material was collected for our preliminary paper. Detailed questionnaires to patients were employed to obtain the family histories used for our pedigrees. The questionnaires asked for specific facts concerning each member of the family. When there was any doubt as to an answer, letters were written about specific points, and in many cases a series of letters was sent to a single individual. Since the patients treated by the clinic are of a high type of intelligence (see below), the information obtained is looked upon as very trustworthy.

We realized at the outset the necessity of getting full pedigrees of both maternal and paternal sides of the families. The work was done as far as possible with methods commonly employed in genetics. This is in contrast with methods sometimes used in studies of this nature in which records are kept only of the affected parents of the individuals being studied, omitting the contribution to the inheritance made by the parent who may not happen to show the character. Of this latter nature are many of the charts referred to by BAUR, FISCHER, LENZ including the pedigrees relating to allergy from GÄNSSLEN, UNGER, CROWDER, ULLMAN, and STUDY.

Data as complete as could possibly be obtained were collected from fifty-five families, both maternal and paternal sides of each pedigree being included. Forty-one of these pedigrees gave a sufficiently complete history for genetic use. We have retained only the pedigrees which gave fairly complete information about two and usually three generations. Most of

the people to whom questionnaires were sent have suffered from migraine. The preliminary report concerning hay fever was based upon a study of twelve families. Including the pedigrees of the earlier paper, therefore, fifty-three out of sixty-seven different family histories complete enough for genetic study form the basis of this report.

In the preliminary report we found that hay fever and other forms of allergy may act as a dominant character but that their dominance is irregular, for it is often incomplete and sometimes an unaffected individual may transmit the ability to become sensitive to several or to all of his or her children. In the earlier paper the various forms of allergy were considered as different expressions of a single gene, although the possibility of multiple factors as a cause was not overlooked. The genetic evidence then available led to this conclusion, but it is to be noted that it was quite in harmony with the clinical behavior of the diseases.

After studying allergic families for several years we have found that if allergy is present in a strain, it is extremely rare (families 2 and 23) for a generation to occur without some member of the family being affected. We have only five cases of this kind. Unaffected individuals may pass on the allergy to their children, but usually these individuals have allergic brothers or sisters even though they themselves have no allergic diseases. In no case which we have studied does allergy skip two generations; that is, allergic individuals have never been found to appear after the family has been negative for two generations. Occasionally one generation may be entirely skipped, but we have no record of two negative generations followed by a third allergic one, and we have sought carefully for cases bearing upon this point. For these reasons we have come to regard a family as incapable of transmitting the gene for sensitivity if in our pedigrees it has been entirely negative for two generations, or if the parental generation and the two grandparents also are negative.

The changing or incomplete dominance of allergy may best be explained by quoting a couple of paragraphs from our earlier paper:

"The time of appearance of the sensitivity in the ontogeny of the individual differs greatly in different people. In some cases a child develops a sensitivity very shortly after birth and in other cases individuals who have never before had trouble develop a sensitivity at the age of seventy. Between these two extremes are a whole series of ages, at any of which a sensitivity may develop. Consequently, if an individual belongs to an affected family and has had no trouble, there is still a great probability that he may later develop it. Thus no chart of an affected family can ever be considered as complete, for those once classed as normal may later develop some form of allergy.

"Another point must also be considered. In order for any form of sensi-

tivity to become manifest there must be, in addition to the sensitivity, the external factor that causes the trouble. A child sensitive to certain forms of protein will not develop urticaria or eczema unless he eats that protein. Similarly an individual sensitive to a certain kind of pollen develops hay fever only when exposed to that pollen. In recent years much has been added to our knowledge of the interaction of heredity and environment. Apparently the study of inheritance of hay fever, etc., reveals another case in which a definite environment as well as a definite inheritance is necessary to produce a definite character."

These statements of our earlier paper apply equally well to migraine. This form of allergy sometimes occurs in young children and on the other hand many people from allergic families develop this difficulty much later in life. The environment necessary to produce allergy in members of an allergic family differs with respect to the different allergies. Hay fever does not develop unless the patient comes in contact with a particular kind of pollen which furnishes the necessary irritation. For example, a patient born with the ability to become sensitive, who comes in contact with Russian thistle pollen develops a sensitivity to Russian thistle and may have hay fever symptoms on adequate contact. Likewise, those who live in the timothy section of the United States and who come in adequate contact with timothy pollen have developed a sensitivity to timothy and have hay fever symptoms from contact with timothy pollen. Similarly migraine, eczema, etc., develop only when the foods to which the individual is sensitive are eaten. Both the pollen in the one case and the particular food in the other may be regarded as the environment necessary to bring out the allergy.

The data upon which these conclusions in regard to migraine are based are less open to criticism than in the case of hay fever on the score of the environmental condition necessary to bring out the sensibility. That is, there is less possibility that the negative cases are not truly negative but have merely not happened to meet the proper environment to cause the allergy to manifest itself. The reason for this is that with the normal varied diet which most adults enjoy there is little chance that a person can escape the sensitizing protein long after reaching adulthood.

That both a definite heredity and a definite environment are necessary to produce a given character is well known and many cases of both animals and plants can be cited to support this principle. The early work of BAUR on the effect of temperature on the color of the primrose was perhaps the first case of this kind noted. Since that time modern work has shown that many adult characteristics are dependent on a special environment as well as on a special gene for their fulfillment. MORGAN'S work on abnormal abdomen in Drosophila showed that this character is largely dependent upon moisture. IRA WILSON'S hereditary tumor in Drosophila is dependent to a

large extent upon the amount of food. The work of the senior author on extra legs in Drosophila (HOGE 1914) has shown that this character appears only when the conditions of temperature are suitable. Similarly KRAFKA has demonstrated that the number of facets of "bar eye" in Drosophila varies in relation to temperature. Also there are many examples to show that age may operate to make manifest the determining factor in various characters. Pink-eyed flies have light eyes when young, but when old their eyes are almost as dark as those of the wild red fly. Black-bodied flies when young are as light colored as the normal fly and they develop black pigment only with age. These examples are sufficient to show that

Key to Charts

female

male

allergic individuals

on known facts concerning individual

individuals known to be negative

individuals who died young

individual considered negative, but data incomplete

family history incomplete as to members; except in cases so marked, every individual in every family is listed

allergic, but type unknown

individuals from families with no allergy

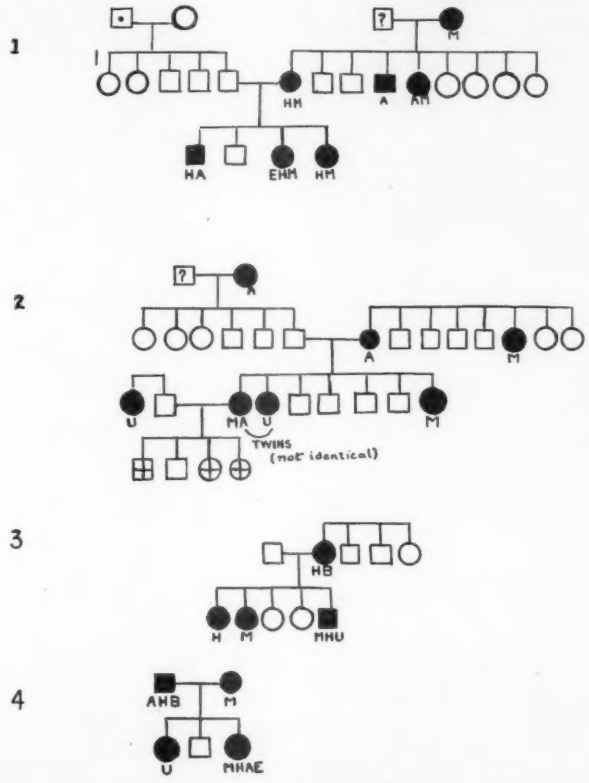
negative individuals from families known to be allergic

A Asthma
B bronchitis
C colitis
E eczema
H hay fever
M migraine
U urticaria

both environment and heredity are determining factors in the development of a particular individual. Studies on the inheritance of allergy show that with it, just as with these cases of the lower animals and plants, an individual does not become allergic unless the environment to which he or she is subjected is such as to bring out the latent sensitivity.

An interesting fact early observed in this study was that allergic persons commonly marry into allergic families. This is still true even in the cases where one or both of the individuals concerned are themselves negative. Our records include only a few cases in which marriage occurs between a person from an allergic family and one from a negative line. The explanation for this fact can only be conjectured. Allergic families have

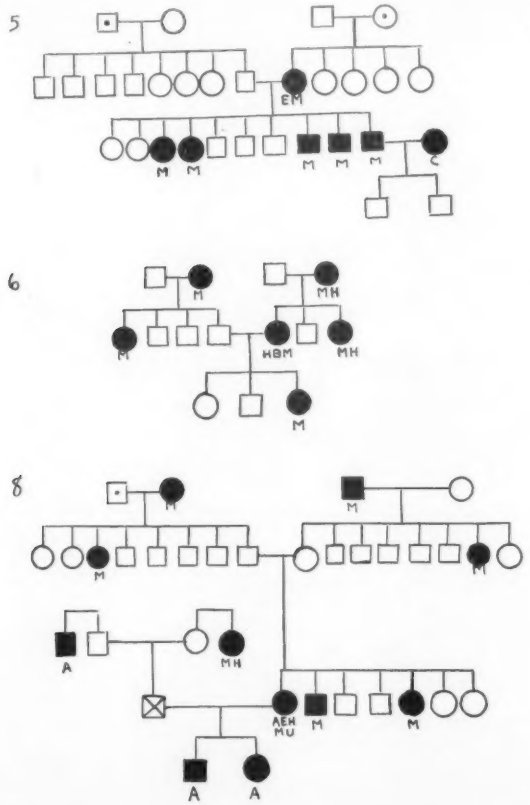
been found to be usually of a high grade of intelligence, and, as BALYEAT (1929) has shown, a hay fever sufferer customarily has a higher intelligence quotient than does the average person. It may be surmised that this fact has something to do with the frequent marriages between allergic families and that in some cases their higher intelligence serves as a mutual attraction. Since allergic persons make up only a small percentage of the entire



population, their frequent marriage is worthy of note, although no means is at present known which favors the mating of these persons.

In our paper on hay fever we have stated that some families show only one form of allergy. It is seldom, however, that any family is specific for one allergy alone. Only one of the twelve families of our earlier paper showed no allergy but hay fever. One had only asthma and hay fever, and the other ten had in addition either eczema, bronchitis, urticaria, migraine, or a combination of several of these allergies.

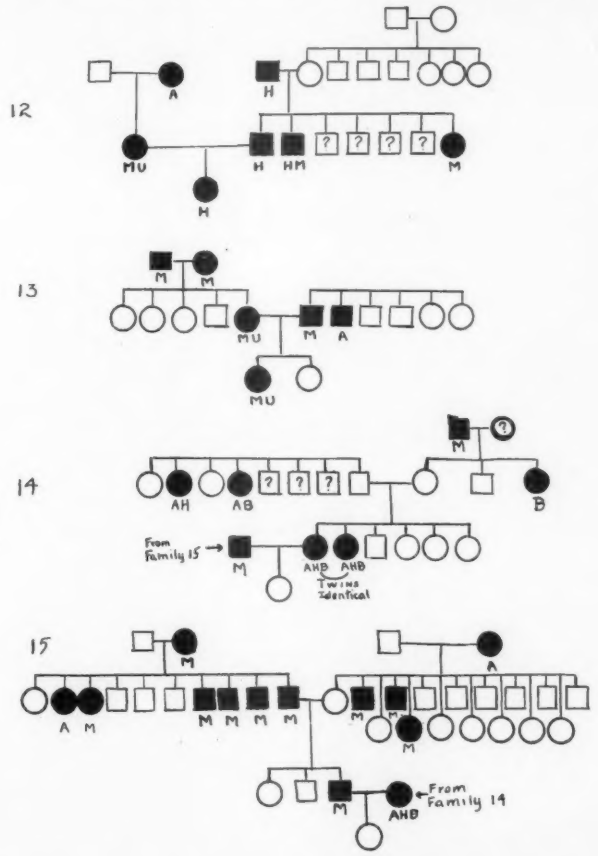
Our present study of forty-one families shows similar conditions. Only five (for example families 22, 23, 27, 37) exhibit branches which before crossing with other allergic families show nothing but migraine, and in most of the families there are various combinations of the different allergies, a single individual sometimes suffering from four to five types (example, family 8). In eleven families there are two kinds of allergy and in twenty-six there are three or more.



Since in the families from whom we have collected data so many forms of allergy are present, and since allergics commonly marry individuals from similar families, it is extremely difficult to determine positively the number of genetic factors that cause the different forms of sensitivity.

In our present study we have endeavored to ascertain whether our pedigrees could be explained as due to the operation of several genes acting

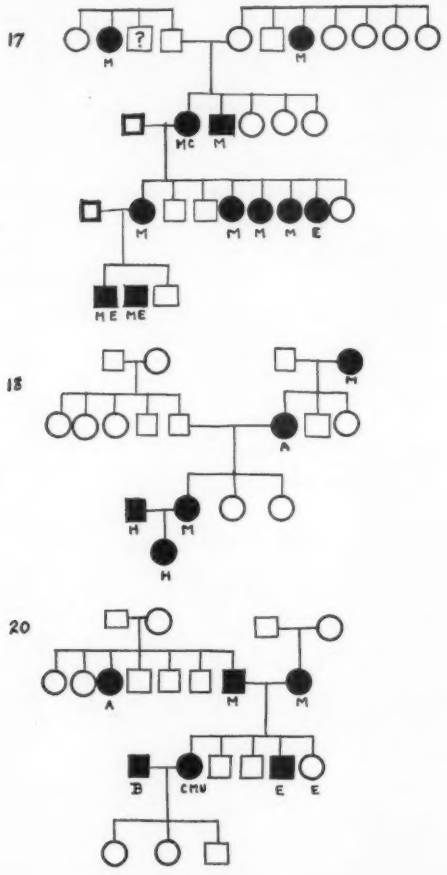
together, perhaps one gene being necessary for an individual to become allergic and a special modifying gene being necessary to produce each type of allergy. This possibility, mentioned in our previous paper, has been suggested by several critics, but the number of pedigrees previously considered was insufficient to settle the point.



In order to gain some light on this matter we have examined carefully the pedigrees of those families in which an allergic individual marries into a negative line. As already stated, a line is considered negative when for two generations no member has exhibited an allergy or when the parental generation and the two grandparents also are negative. The study of this type of mating has proved the most productive phase of our research.

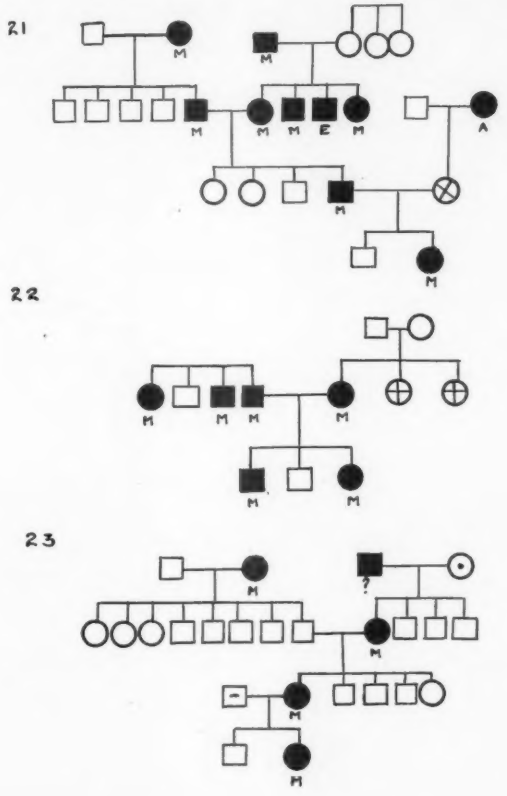
Obviously a cross of this kind will show at once what types of allergy are dominant, for if any allergy appears again in the F_1 from such a cross its dominance is certain.

Unfortunately, as we have stated before, marriages of this type are not numerous. We have among our fifty-three pedigrees twelve matings of this



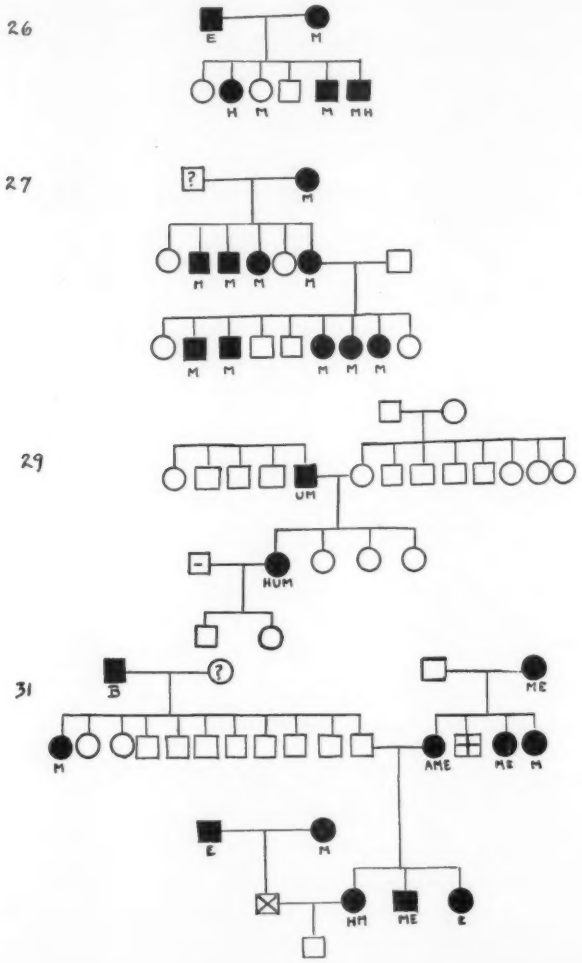
type (Nos. 1, 5, 12, 18, 29, 32, 34, 37, 38, 41, 42, 43). In pedigree 1, hay fever and migraine act as dominants, in No. 12, hay fever; in No. 29, urticaria and migraine, in No. 34, eczema, and in No. 43, asthma acts in a similar manner. Thus each of these five types of allergy may show dominance.

However, in spite of this dominance, not even half of the F_1, as we should expect if the allergic parent were heterozygous, develop the sensitivity. In family 29 a man affected by migraine and urticaria married into a negative family and had four children; one of the four had hay fever, urticaria and migraine, but the other three were negative. In family 32, a woman with migraine and hay fever married into a negative line and



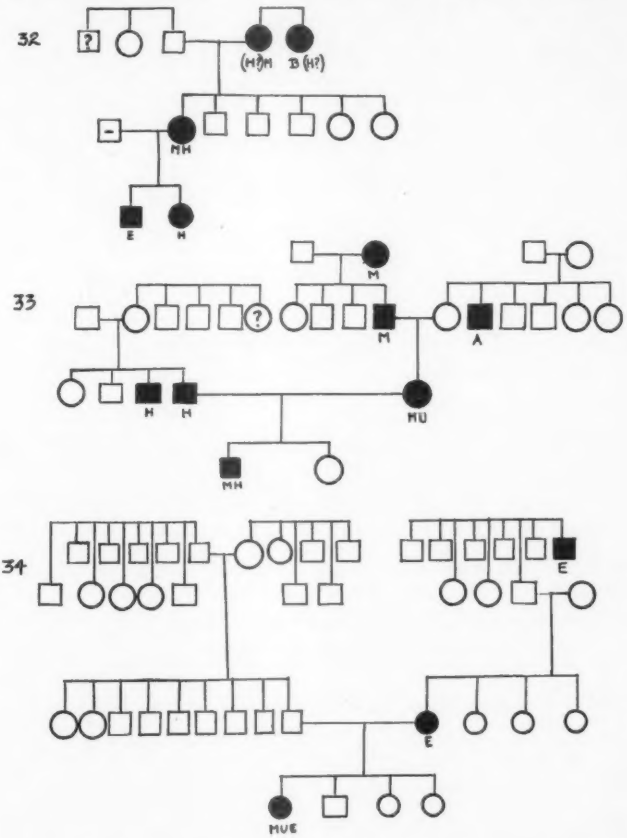
had two children, one of whom had eczema, the other hay fever. In family 18, a woman with asthma married into a negative line and had a daughter with migraine. In family 38 a man with hay fever and asthma married into a negative line and had nine children, of whom only one daughter had any trouble—hay fever and eczema. In family 34 a woman with eczema married into a negative line and had four children only one of whom showed any allergic trait, and this child had migraine, urticaria, and ec-

zema. In family 41 a man with migraine married into a negative line and had six children, four of whom were allergic, a son and a daughter each having hay fever, a son migraine, and a daughter urticaria and eczema.



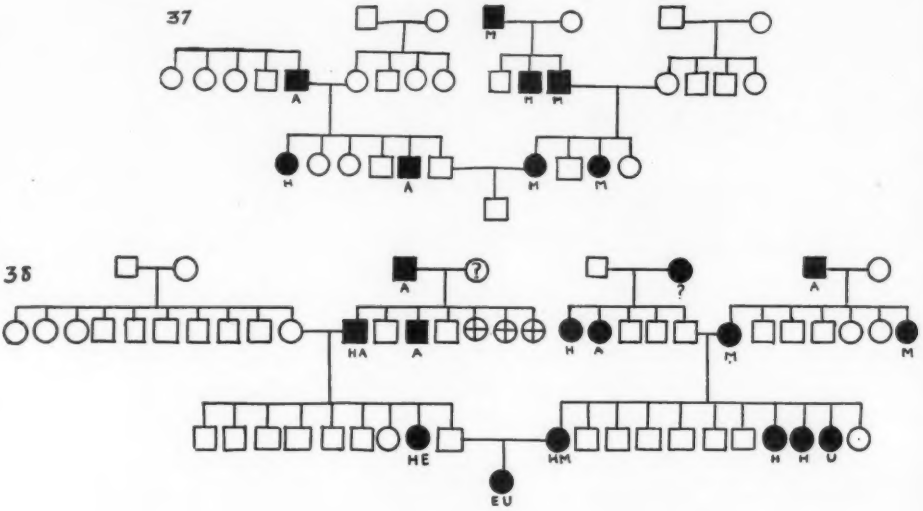
If we consider sensitivity as due to one gene and each allergy as due to a modifying gene, then we must assume that in each of these cases the negative line brought in the special modifiers necessary to produce the different types of allergy. If these modifiers exist, then, as has just been

shown, they must act as dominants when present in connection with the gene for sensitivity. Since these possible modifiers are dominant, the negative lines referred to above would have to bring in one or more dominant modifying genes for the different forms of allergy, these modifying genes being unable to express themselves without the gene for sensitivity which is lacking in the negative line. Following out this line of reasoning and assum-



ing that there is a specific modifying gene for each allergy, we would expect these dominant modifiers to cause new allergies in a fair percentage of the children. If the negative parent were heterozygous for the modifying gene, only half the children would have the new allergy. In any event we should never expect complete dominance, since we have shown that the dominance is variable. However, the new combinations in the F_1 are of various

kinds, one individual having one new allergy, another a second, and still others having different combinations of them. Sometimes only a small percentage of the progeny show the dominance of a particular type of allergy, and in other cases the same allergy that has been shown in similar crosses to act as a dominant does not appear in the F_1 although it may be present in the parent.

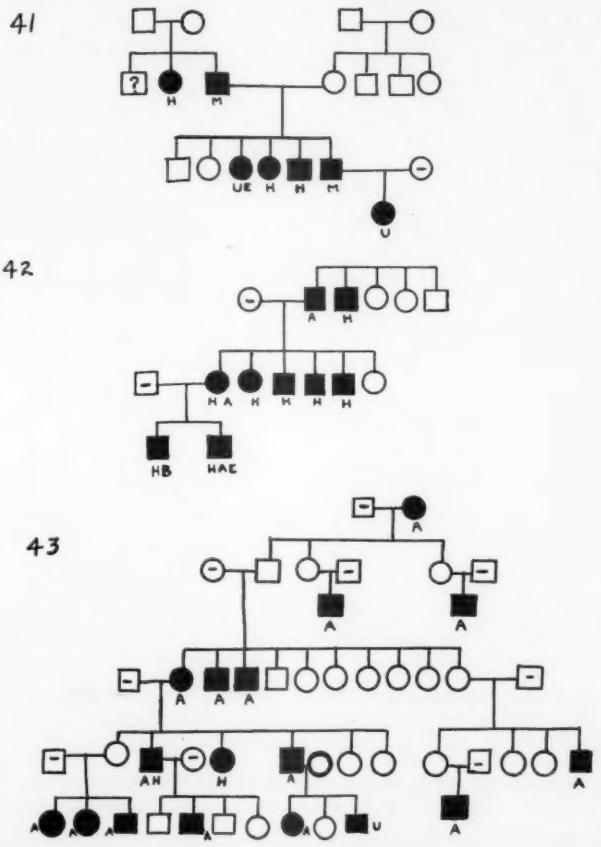


Another fact revealed by this type of mating is that a person with one form of allergy may transmit other forms to his or her children. Examples to illustrate this condition are to be found in many pedigrees and are of two kinds: Either the allergy not present in the parent may have been

FAMILY NUMBER	NEGATIVE LINE CROSSED WITH	ALLERGY IN PARENT FAMILY BUT NOT IN PARENT	ALLERGIES IN PARENT AND ALSO IN F_1	ALLERGIES OF PARENT FAMILY ALSO IN F_1	NEW ALLERGY IN F_1
1	MH	A	MH	A	E
5	EM		M		
12	H		H		M
18	A	M		M	
29	UM		UM		H
32	MH		H		E
34	E		E		MU
38	HA		H		E
41	M	H	M	H	UE
42	HA		HA		BE
43	A		A		H

observed in other members of his family, or in other cases some form entirely new to the strain may appear.

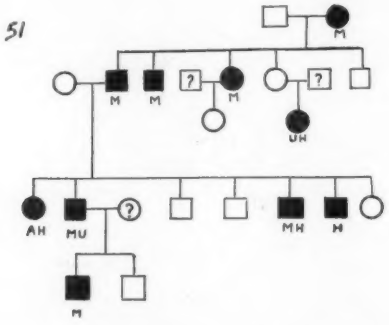
A summary of the results of some of the matings between allergic and negative families will make these facts more evident. (Note: A-asthma; B-bronchitis; E-eczema; H-hay fever; M-migraine; U-urticaria.)



As this chart shows, the allergy of the parent may not appear at all in the F_1, although in other cases this same allergy acts as a dominant. Since the person from the negative line, after crossing with the allergic line, produces various combinations of allergies, some present in the allergic parent, some present in members of the family of the allergic parent, and others entirely new in the family, and since these allergies, old and new to the family, do not appear in any regular proportion, it seems illogical

to consider that the negative line has brought in modifying factors for a new set of allergies. If such were the case these new allergies, being dominant as we have shown, should appear in a fairly large proportion of the F_1, a result not found to be true.

Matings between allergic families also establish this point. In family 8, migraine had appeared for two generations on both sides and no other allergy had been manifest. The father and mother, both free from allergy, had seven children, two of whom suffered from migraine, and another from five allergies, namely, migraine, eczema, asthma, urticaria, and hay fever. How was it possible for four allergies, apparently new to both sides of the family (three of which have been shown in our other crosses with negative lines to be dominants) to be present for two generations without manifesting themselves?



Other matings of two individuals, both from allergic families, also produce children with allergies which have not appeared in either family. Thus in families 2, 20, and 13 urticaria appears as a new allergy; in family 17 eczema; in family 31 hay fever. In this last case, as in the cases with negative lines, two allergies present in the family did not appear in any of the progeny. One parent had asthma and a grandparent had bronchitis, neither of which reappeared in the children.

These facts all lend support to the theory that there is only one gene for sensitivity and that this gene expresses itself in various ways in different individuals. This point, we believe, is definitely established by our crosses with negative lines. We must admit, however, that we have no explanation to offer for the five pedigrees in which there is only one allergy. It is of course possible that some of the individuals in these pedigrees may develop other allergies later in life.

We have one case of identical twins in family 14. Both of the twins have the same three forms of allergy. It is probable that the environment of the twins is very similar which may account for the occurrence of the

same allergies. Obviously, however, no conclusion can be drawn from one case and we shall hope to find other allergic identical twins.

In reviewing our charts, it is at once evident that migraine is more prevalent in females than in males. Among our pedigrees we find 64 males with migraine and 126 females so affected. At first sight, it might seem that migraine has something to do with the sex chromosome, for if it were dominant, as we have shown, but also sex linked, we should expect more migraine females than males. Our records show, however, that a son may inherit migraine from either his father or his mother. He may have migraine if his mother is from a negative family (family 41) and his father has migraine; or if his father is from a negative family and his mother has migraine (family 5). Apparently a son may be allergic if his parents are from allergic lines, regardless of which parent has migraine, or even if neither has that special form of allergy. The following chart makes this matter clear. In these cases both father and mother are most probably from allergic families. (Note: See p. 134, for meaning of letters. [..] no allergy.)

SON	FATHER	MOTHER	FAMILY
M	..	M	15, 17, 21, 51
M	..	MEA	31
M	M	..	51, 21
M	H	MU	33
M	E	M	26
M	..	HB	3
M	..	..	8, 17
M	..	A	15
M	M	M	21, 22

It has been suggested that allergy might be due to a factor which would bring it about in females when in the heterozygous condition, but that the homozygous presence of the gene would be necessary to cause the allergy to appear in the male. A condition just opposite to this exists in cattle where if the gene for "hornless" is present in the duplex condition in a female she is hornless, whereas only the simplex condition is necessary to make a hornless male. The inheritance of migraine obviously is not comparable to this case, for a male may develop migraine when he inherits the gene for sensitivity from only one side, that is, when he is heterozygous. Since our records show that with the same inheritance females are more likely to have this allergy than males, the reason for this difference must probably be sought in the physiological differences beween the sexes. One might conjecture that the hormones related to sex may be the cause of the different proportions.

The conclusions of this paper are in harmony with the work of most other students of the inheritance of allergic diseases. Except for the works

of ADKINSON and BUCHANAN the types of allergy have been considered as due to dominant factors. ADKINSON'S results can be explained as due to a dominant gene, dependent for its expression to some extent upon the environment. Migraine has been regarded as a sex-linked character by several writers because of the large number of females who have it. However, we have shown that migraine may be inherited by the son from the father when the mother's line is negative and therefore cannot be sex-linked. Also migraine is interchangeable with the other allergies and acts as the expression of the same gene for sensitivity which obviously has no connection with sex. Since allergic women are apt to suffer from these headaches during the menstrual period (a fact which has been referred to a number of times in the literature and which we also have observed), the preponderance of migraine females can best be explained as correlated in some way with the sex hormones and with physiological processes. Finally, we have found no connection (as did STUDY and GÄNSSLEN) between gout and the allergic diseases mentioned.

We have shown that any allergy may act as a simple dominant, and an allergic individual may transmit either the same or another type of allergy. There is thus no regularity in the transmission of the different types of allergy and the individual known to carry the gene for sensitivity may be normal. Our evidence thus shows that most probably allergy is not due to multiple factors but rather to the expression of a single gene for sensitivity which manifests itself in different ways, depending to some extent upon the environment of the individual. The gene for allergy is not sex linked. The greater number of migraine females cannot be explained as due to a sex-linked gene.

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INHERITANCE IN BARLEY[1]

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INTRODUCTION

In recent years, considerable information on inheritance in barley has been obtained. At present, some five or six different linkage groups have been found. Various factors have been located in the different groups but few have been mapped. The present studies involve several factor pairs which previously have not been located in any of the groups studied.

REVIEW OF LITERATURE

The review of literature on the genetics of barley is confined to the characters studied in this paper.

Chlorophyll deficiencies

Colsess V described by ROBERTSON and DEMING (1930) is a chlorophyll deficient plant which grows to maturity but is less vigorous than the normal green type. The plant color according to RIDGEWAY, plate XVII, is dull green yellow. The chlorina seedlings are recessive to normal green.

The interrelationship of the factor pair ($F_c f_c$) for green *versus* chlorina seedlings in Colsess V has been tested (ROBERTSON and DEMING 1930) with the following factor pairs: (Kk) for hoods *versus* awns in linkage group IV, ($A_t a_t$) for green *versus* white seedlings in Trebi found in linkage group II, (Ff) for green *versus* chlorina seedlings in Minnesota 84–7 located in linkage group I (ROBERTSON, DEMING and KOONCE 1932).

Inheritance of other characters

A rather intensive review of the inheritance of the following factor pairs is given by DAANE (1931). Hulled *versus* naked, long *versus* short haired rachilla, hoods *versus* awns, and six-row *versus* non-six-row all segregated in simple Mendelian ratios. He also summarizes the published data on six-row *versus* non-six-row. The Vv factor differentiates between six-row and non-six-row but the intermedium factor pair Ii influences the non-six-row head type in F_2 and F_3. He concludes that "Defeciens," "distichon," and

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"vulgare" form an allelomorphic series but that intermedium or low fertility reported by HARLAN and HAYES, GRIFFEE, and ROBERTSON is not an allelomorph of the above mentioned series.

MATERIAL AND METHODS

This paper presents studies of the inheritance of simple Mendelian factor pairs and their possible linkage relations. The following symbols were used to designate the different factor pairs studied.

Non-six-row *versus* six-row	*Vv*
Black *versus* white glume color	*Bb*
Hulled *versus* naked caryopsis	*Nn*
Hoods *versus* awns	*Kk*
Intermedium *versus* non-intermedium	*Ii*
Long *versus* short haired rachilla	*Ss*
Green *versus* white seedlings	*Aa*
Green *versus* yellow seedlings	*Xx*
Green *versus* Chlorina seedlings	*Ff*
Purple *versus* white straw color	P_r p_r

The parental varieties were differentiated by the following factor pairs:

TABLE 1

List of factor pairs found in the different parents.

NEPAL	CHEVALIER	BLACK HULLESS	TREBI	COLSESS V	NILSSON-EHLE NO. 2
vv	*VV*	*vv*	*vv*	*vv*	*VV*
..	..	..	$p_r p_r$	$p_r p_r$	$P_r P_r$
bb	*bb*	*BB*	*bb*	*bb*	*bb*
nn	*NN*	*nn*	*NN*	*NN*	*NN*
KK	*kk*	*kk*	*kk*	*KK*	*kk*
..	*ii*	..	*II*	*II*	..
SS	*SS*	*SS*	*ss*	*ss*	*SS*
$X_n x_n$	..	$A_b a_b$	..	$f_c f_c$	$A_2 a_2$

Methods of conducting experiments

Methods similar to those described in a previous publication (ROBERTSON 1929) were used. The same symbols were used to interpret characters as those used in previous publications (ROBERTSON 1929, ROBERTSON and DEMING 1930, ROBERTSON, DEMING and KOONCE 1932, DAANE 1931). Additions to the latter list were made for the following character pairs: Intermedium *versus* non-intermedium (*Ii*) and Purple versus white straw color (P_r p_r).

INHERITANCE OF SIMPLE MENDELIAN FACTOR PAIRS

In the cross between Colsess V and Chevalier, the parents apparently differed by two pairs of factors for fertility of the lateral florets and the

results were entirely similar to those obtained by HARLAN and HAYES in 1920. The genotypes of the F_2 plants were determined by breeding behavior in F_3, the particular purpose being to find which of the F_2 plants carried the intermedium factor. Table 2 gives the type of segregations which were obtained. The F_2 genotypes belonging to classes 4 and 7 evidently carried the intermedium factor in a recessive condition. The remaining classes had the dominant condition for the intermedium factor. Class 1 which bred true for six-row could not be used in this material, as it could not be differentiated for the presence or absence of the intermedium factor. Using the material in classes 2 to 7, a ratio of 1184 F_2 plants carrying dominant *I* and 447 of the recessive type were obtained. These classes were used later in the study of the interaction between the chlorina factor pair and the intermedium factor pair.

TABLE 2

F_3 segregating classes and F_2 genotypes of non-six-row plants from a cross Colsess V (vulgare)×Chevalier (distichon).

CLASS	F_3 SEGREGATING CLASSES	F_2 GENOTYPE
2	6-row and intermedium	*VvII*
3	6-row intermediate, intermedium and 2-row	*VvIi*
4	6-row and 2-row	*Vvii*
5	Homozygous for intermedium	*VVII*
6	Intermedium and 2-row	*VViI*
7	2-row	*VVii*

The inheritance of the character pair green *versus* white seedlings was studied in a cross between Colsess×Black Hulless and Black Hulless×Trebi. A simple Mendelian segregation was obtained (table 3).

The inheritance of straw color was studied in a cross between Nilsson Ehle No. 2 and Trebi. The data in table 3 indicate that purple *versus* white straw color is the result of the interaction of a simple Mendelian factor pair.

TABLE 3

Inheritance of simple Mendelian characters.

FACTOR PAIRS	CHARACTERS	*A*	*a*	DEV. IN NUMBERS	DEV. P.E.
Ii	Intermedium *versus* non-intermedium	1184	447	39.25	3.3
A_{ya_b}	Green *versus* white seedlings	1350	428	16.50	1.3
$P_r p_r$	Purple *versus* white straw	676	217	6.25	0.7

INTERRELATION OF SIMPLE MENDELIAN FACTOR PAIRS

The factor pair ($F_c f_c$) and its relationship with the following linkage groups III (*Nn*), IV (*Kk*) and V (*Ss*) (DAANE 1931), (ROBERTSON 1932) was studied.

Green versus chlorina seedling (F_cf_c) and covered versus naked caryopsis (Nn)

The interrelationship of the factor pair (F_cf_c) and (Nn) was studied in a cross between Colsess V and Nepal. The number of chlorina plants was lower than expected on the basis of a segregation for 3 green to 1 chlorina. The χ^2 test for independence was used. The data presented in table 4 indicate that the factor pairs (F_cf_c) and (Nn) are inherited independently of each other.

Green versus chlorina seedlings (F_cf_c) and the intermedium versus non-intermedium (Ii)

The intermedium and non-intermedium classes in table 3 were used for the study. They were regrouped for plant color, and a close fit to a 9:3:3:1 ratio was obtained (table 4). The data presented indicate that the factor pairs (F_cf_c) and (Ii) are inherited independently of each other.

Green versus chlorina (F_cf_c) and long versus short haired rachilla (Ss)

The interrelationship of the factor pairs (F_cf_c) for green *versus* chlorina seedlings and the factor pair (Ss) which is located in group V was studied in a cross between Colsess V and Nepal 1. The observed and calculated ratios for independence are given in table 4. The χ^2 test indicates that the factor pairs (F_cf_c) and (Ss) are inherited independently of each other.

The data already presented show that the factor pair (F_cf_c) for green *versus* chlorina seedlings in Colsess V is inherited independently of factors found in the following groups: Group I (1932); Group II (1930); Group III (Present paper); Group IV, (1930) and Group V (1932). The factor pair (F_cf_c) is, however, linked with the factor pair (Y_cy_c) for green *versus* virescent seedlings in Coast III (1932).

Intermedium versus non-intermedium (Ii) and hoods versus awns (Kk)

The interrelationship of the factor pairs (Ii) for intermedium *versus* non-intermedium and (Kk) for hoods *versus* awns was studied in a cross between Colsess V and Chevalier. The factors went into the cross in the following order: *KKII* in Colsess and *kkii* in Chevalier. The F_2 segregation should, therefore, be in the coupling phase.

In testing the interrelationship of the factor pairs (Kk) and (Ii) the following segregations were obtained (table 5).

If linkage occurred it would be in the coupling phase, since the factor pairs went into the cross as *KKII* and *kkii*. The above data indicate such a linkage. IMMER'S tables for calculating linkage intensities were used and a crossover percentage of 15.12 ± 0.65 was obtained; when the observed

TABLE 4

Character pairs independently inherited.

CROSS	CHARACTERS	PHASE	AB*	Ab	aB	ab	χ^2	P
Colsess V×Nepal 1	$F_c f_c$ Nn	Repulsion Observed	1432.0	446.0	379.0	127.0	0.40	0.53
		Calculated	1426.6	451.4	384.4	121.6		
Colsess V×Chevalier	$F_c f_c$ Ii	Coupling Observed	887.0	339.0	299.0	105.0	4.86	0.18
		Calculated	916.9	305.6	305.6	101.9		
Colsess V×Nepal 1	$F_c f_c$ Ss	Coupling Observed	1392.0	486.0	387.0	119.0	1.17	0.29
		Calculated	1401.4	476.6	377.6	128.4		
Colsess×Chevalier	Vv Kk	Repulsion Observed	1302.0	464.0	405.0	137.0	0.20	0.65
		Calculated	1306.0	460.0	401.0	141.0		

χ^2 Calculated for independence.

* The classes for the character pairs are headed by the general symbols AB, Ab, aB and ab.

TABLE 5

F_2 segregations of a cross between Colsess V and Chevalier. The F_2 classes for the (Ii) factor pair were determined from F_3 segregations.

	HOODED INTER-MEDIUM	HOODED 2-ROW	AWNED INTER-MEDIUM	AWNED 2-ROW	χ^2	P
Observed	1078.0	129.0	106.0	318.0		
Calculated 9:3:3:1	917.4	305.8	305.8	101.9	718.80	very small
Calculated 15.12 percent crossover	1109.3	114.0	114.0	293.8	5.42	.07

ratio is compared with a calculated ratio with 15.12 percent crossing over, a close fit was obtained.

Since there is a linkage of the factor pairs (*Kk*) and (*Ii*) and it is in the coupling phase, this should prove the genetic constitution of the parents for the (*Kk*) and (*Ii*) factors. Table 5 shows a coupling type of linkage and, therefore, (*KK*) and (*II*) must have gone into the cross in Colsess since it is hooded, and *kkii* must have gone into the cross in Chevalier since it is awned and non-intermedium. In a previous publication, ROBERTSON (1929) obtained a ratio of 1 six-row, 2 intermediate and 1 two-row in a cross between Colsess I and *H. distichon nigrinudum*. The two-rowed parent evidently has the genetic constitution (*VVII*) and Colsess I has the genetic constitution (*vvII*). In this case the only factor pair segregating would be (*Vv*) and a simple Mendelian segregation would be obtained in F_2, proving the constitution of *H. distichon nigrinudum* to be (*VVII*), since Colsess has already been shown to have the (*II*) factor in the dominant condition. As has already been stated of the cross Colsess V and Chevalier, classified as *H. distichon* by HARLAN (1918), seven classes were obtained in F_2 showing that two factor pairs are involved: the (*Vv*) factor pair for non-six-row *versus* six-row and the (*Ii*) factor pair for intermedium *versus* non-intermedium. Since the Colsess parent has the genetic constitution (*vvII*) the genetic constitution of Chevalier would be (*VVii*) giving a F_1 plant which would be heterozygous for both factor pairs. Since two types of segregation were obtained from crosses of Colsess × *distichon* the indications are that the (*Ii*) factor pair is independent of the allelomorphic series for six-row, "*distichon*" and "*deficiens*."

From the same crosses where (*Ii*) and (*Kk*) indicated a linkage the interrelationship of the factor pairs (*Kk*) and (*Vv*) were studied also. For this purpose, the F_3 data for rows were again used. The groups were classed as follows: six-rowed hooded; six-rowed awned; non-six-rowed hooded and non-six-rowed awned. Table 4 gives the different segregating groups for the factor pairs (*Vv*) and (*Kk*). From the data obtained it may be concluded that the factor pairs (*Vv*) and (*Kk*) are inherited independently of each other.

Since there is no indication of linkage between the factor pairs (*Kk*) and (*Vv*) it is also evident that the factor pairs (*Vv*) and (*Ii*) are in different linkage groups. This is further borne out by earlier studies which show (*Vv*) and (*Kk*) to be located in different groups (ROBERTSON 1929, DAANE 1931).

Interrelation of green versus white seedlings ($A_b a_b$) and long versus short haired rachilla (Ss)

The interrelationship of the factor pair ($A_b a_b$) with (*Kk*) (*Nn*) and (*Ss*) was studied in crosses between Colsess × Black Hulless and Black Hulless × Trebi. In one of the former crosses only the Black Hulless parent was heterozygous for white seedlings and the relationships of the factor pair ($A_b a_b$) were studied in this cross. The following simple Mendelian segregations were obtained:

TABLE 6

Inheritance of simple Mendelian characters. F_2 plants heterozygous for factor pair ($A_b a_b$).

	CHARACTER	*A*	*a*	DEVIATION IN NUMBERS	DEVIATION P.E.
Kk	Hoods *versus* Awns	335	115	2.5	0.40
Nn	Covered *versus* naked	331	119	6.5	1.05
Ss	Long *versus* short haired rachilla	300	150	37.5	6.05
**Ss*	Long *versus* short haired rachilla	683	216	8.75	1.00

* The families giving this segregation were homozygous for green.

In table 6 it will be seen that long *versus* short haired rachilla does not fit the calculated 3 to 1 ratio very well. The families from which the first segregation is obtained were also heterozygous for seedling color. The second count which fits a calculated 3:1 ratio was obtained from two pure green families. This would indicate that the character long *versus* short haired rachilla is due to the interaction of a simple Mendelian factor pair, but the segregation is influenced by the presence of the factor pair ($A_b a_b$) for green *versus* white seedlings.

The other two factor pairs (*Kk*) and (*Nn*) seem to fit the calculated 3:1 ratio very well. Since the factor pair ($A_b a_b$) was also present in the families in which the above characters were studied and has no influence on the segregation one can conclude that the factor pairs (*Kk*) and (*Nn*) are inherited independently of the factor pair ($A_b a_b$).

A further study of the interrelationship of the factor pairs ($A_b a_b$) and (*Ss*) was made in a cross between Black Hulless and Trebi.

The F_2 data when studied in relationship to the two factor pairs show a poor fit to a calculated 9:3:4 ratio which is similar to the data found in the Colsess cross.

TABLE 7

F_2 segregation of long versus short haired rachilla and green versus white seedlings.

CROSS		GREEN LONG	GREEN SHORT	WHITE	χ^2	P
Colsess × Black Hulless	Observed	300	150	149	16.7	0.0003
9:3:4	Calculated	336.9	112.3	149.8		
Trebi × Black Hulless	Observed	973	483	517	52.58	very small
9:3:4	Calculated	1109.8	369.9	493.3	..	..

Since long haired rachilla went into the cross in the heterozygous parent a decrease in the number of long haired rachilla plants would be expected if the factors for white seedlings and long haired rachillas were linked. COLLINS' formula for use when one of the classes is missing was used to determine the crossover percentage.

$$p = \sqrt{\frac{AB - 2Ab}{AB - Ab}}$$

In the Colsess × Black Hulless cross no crossing over was obtained. In the Trebi × Black Hulless cross a value of 6.93 percent was obtained. The fit of the observed to calculated ratio is given for the Trebi × Black Hulless in table 8.

TABLE 8

Observed and calculated ratios for 0 and 6.93 percent crossing over as determined from F_2 data.

CROSS	GREEN LONG	GREEN SHORT	WHITE	χ^2	P
Colsess × Black Hulless Observed	300	150	149	..	..
Calculated 0 percent crossover	299.50	149.75	149.75	.005	very large
Trebi × Black Hulless Observed	973	483	517	..	..
Calculated 6.9 percent crossover	988.9	490.9	493.2	1.53	0.48

Since F_2 data where only three classes are available give results which are considerably less reliable than F_2 data with four classes distinguishable, the progeny of the green plants with long haired rachillas were grown in F_3, and the F_2 genotypes determined for the factor pairs (Ss), long *versus* short haired rachillas and ($A_b a_b$) green versus white seedlings. Table 9 gives the results obtained from the F_3 data.

TABLE 9

Proportion or number of F_2 genotypes as determined from F_3 breeding behavior.

CROSS	*AASS*	*AASs*	*AaSS*	*AaSs*	χ^2	P
Colsess × Black Hulless Observed	3	53	53	180	..	..
Calculated 1:2:2:4 ratio	32.1	64.2	64.2	128.4	51.02	very small
Trebi × Black Hulless Observed	56	319	260	1017	..	..
Calculated 1:2:2:4 ratio	183.6	367.1	367.1	734.2	235.1	very small

The F_2 data further indicate that the factor pairs (Ss) and ($A_b a_b$) are linked.

When the F_2 genotype classes are tested for linkage a crossover value of 23 percent for the Colsess cross and 26 percent for the Trebi cross is obtained.

TABLE 10

F_2 genotype classes and calculated ratios with 23 and 26 percent crossing over.

CROSS	*AASS*	*AASs*	*AaSS*	*AaSs*	χ^2	P
Colsess×Black Hulless	3	53	53	180	..	..
Calculated 23 percent crossover	7.45	49.86	49.86	181.8	3.07	0.38
Trebi×Black Hulless	56	319	260	1017	..	..
Calculated 26 percent crossover	54	307.4	307.4	983.2	8.98	0.02

The P value for the Trebi×Black Hulless cross is small and the high χ^2 value is caused by the *AaSS* group. This discrepancy may have been due to slight errors in classification and some of the plants from the *AaSS* class may have been placed in the *AaSs* class. However, the observed fits the calculated ratio with 23 percent to 26 percent crossing over much better than a ratio calculated for independence of the factor pairs ($A_b a_b$) and (Ss).

When we test the fit of the F_2 data to calculated ratios with 23 percent and 26 percent crossing over, a good fit is obtained also.

TABLE 11

F_2 segregation for long versus short haired rachillas and green versus white seedlings.

CROSS	GREEN LONG	GREEN SHORT	WHITE	χ^2	P
Colsess×Black Hulless Observed	300.00	150.00	149.00	..	..
Calculated Ratio 22.75 percent crossover	307.25	142.00	149.75	0.63	large
Trebi×Black Hulless	973.00	482.00	517.00	..	..
Calculated Ratio 26 percent crossover	1019.80	459.90	493.30	4.44	0.11

It is apparent that the factor pairs ($A_b a_b$) and (Ss) are linked with about 26 percent crossing over.

Purple versus colorless straw (P_r p_r) and non-six-row versus six-row (Vv)

The interrelationship of the factor pair for six-rowed *versus* non-six-rowed and straw color was studied in a cross between the selection Nilsson-Ehle No. 2 and Trebi. There was some indication of linkage. Colored straw and two-rows went into the cross in selection No. 2 and colorless straw and six-row went into the cross in Trebi.

In studying the interrelationship of straw color and rows, F_3 data were used to determine the F_2 genotypes. The results of this study are given in table 12.

The results indicate that the factor pair (Vv) for non-six-row *versus* six-row is linked with factor pair ($P_r p_r$) for purple *versus* colorless straw with about 9.0 percent crossing over.

TABLE 12

F_2 genotypes on the basis of F_3 breeding behavior in a cross segregating for six-rowed versus non-six-rowed and purple straw versus colorless straw compared with a calculated 9:3:3:1 ratio and a ratio calculated with 9 percent crossing over.

	NON-SIX-ROW		SIX-ROW		χ^2	P
	PURPLE	WHITE	PURPLE	WHITE		
Observed	636.00	45.00	40.00	171.00	..	..
Calculated 9:3:3:1	501.75	167.25	167.25	55.75	460.00	very small
Calculated 9 percent crossing over	630.66	38.34	38.34	184.66	2.28	0.52

In order to check the possibility of linkage with the intermedium factor pair, the following F_3 classes were grouped: $2=(II)$, $3=(Ii)$, $5=(II)$, $6=(Ii)$ as intermedium and the other classes, four and seven, were grouped as 2-row. The six-row class had to be discarded as no distinction of the (II), (Ii) and (ii) combinations could be made. The results obtained due to the discarding of the six-row colorless class reduced the number of individuals in the colorless class and gave a ratio which was not a normal 9:3:3:1 ratio but fitted two 3:1 ratios very well.

TABLE 13

F_2 segregation of purple versus white and intermedium versus two-row.

	PURPLE		WHITE		χ^2	P
	INTERMEDIUM	2-ROW	INTERMEDIUM	2-ROW		
Observed ratio	458.0	178.0	34.0	11.0	..	..
Calculated ratio for independence	459.5	176.5	32.5	12.5	0.2668	0.60

The above data further indicate that the factor pairs (Vv) and (Ii) are inherited independently of each other.

SUMMARY

1. The inheritance of the following character pairs was explained on a simple Mendelian basis. Intermedium *versus* non-intermedium (Ii), green *versus* white seedlings in Black Hulless $(A_b a_b)$ and purple *versus* white straw color $(P_r\ p_r)$.

2. The interrelationship of the factor pairs $(F_c f_c)$ for green *versus* chlorina seedlings and factor pairs in linkage groups III, IV and V were studied. (a) The factor pair $(F_c f_c)$ was found to be inherited independently of the following factor pairs: (Nn) Group III, (Kk) Group IV and (Ss) Group V. It has previously been found to be inherited independently of factors in Group I, II and VI.

3. The factor pair (Ii) was found to be linked with the factor pair (Kk) with $15.12 \pm .065$ percent crossing over.

4. The factor pair (*Vv*) was found to be inherited independently of the factor pair (*Kk*), indicating that the factor pairs (*Vv*) and (*Ii*) are not in the same linkage group.

5. The white seedling factor pair ($A_b a_b$) was found to be inherited independently of the factor pairs (*Kk*) and (*Nn*) but was found to be linked with the factor pair (*Ss*) with a percentage crossover of about 26.

6. The factor pair (P_r p_r) was found to be linked with the factor pair (*Vv*) with a crossover value of 9.0 ± 0.68. The straw color factor pair was also found to be inherited independently of the factor pair (*Ii*).

7. The present study adds another linkage group to the six already found. Chlorina ($F_c f_c$) was found to be inherited independently of at least one factor pair in each of the already known groups.

The factor pair (*Ii*) has been added to Group IV.

The factor pair ($A_b a_b$) has been added to Group V and the factor pair (P_r p_r) has been added to Group I.

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INHERITANCE OF COLOR PATTERNS IN THE GROUSE LOCUST *ACRYDIUM ARENOSUM* BURMEISTER (TETTIGIDAE)[1*]

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INTRODUCTION

Studies of inheritance in the grouse locusts (Tettigidae) have hitherto been confined mainly to the more rapidly breeding southern (United States of America) species, *Paratettix texanus* Hancock, *Apotettix eurycephalus* Hancock, *Telmatettix aztecus* Saussure, and *Tettigidea parvipennis pennata* Morse (NABOURS 1929, Bibliography). Four succeeding generations of any of these may be bred in the greenhouse within a year, as against one, or at best, only two generations annually of the more northern varieties and species of this subfamily.

The experiments herein reported were begun in February, 1925, when E. H. INGERSOLL, on an unseasonably warm day, collected some specimens of *Acrydium arenosum* in a wooded area, at the junction of the Kansas and Blue rivers, about two miles southeast of Manhattan, Kansas. Practically all of the specimens used have come from this region. The project was carried on one year by NABOURS, then for one year in cooperation with NELLE HARTWIG (NABOURS and HARTWIG abstract 1926; Master's thesis by HARTWIG 1927, unpublished); then for four years (1927–1931) mainly by IVA LARSON (NABOURS and LARSON abstract 1929; and Master's thesis by LARSON 1929, unpublished). We are much indebted to MARJORIE DEAN for assistance in recording, and to S. FRED PRINCE and RUTH CLENCY for the drawings in the plate.

BIOLOGY OF *Acrydium arenosum*

The species *Acrydium arenosum*, according to HANCOCK (1902), is distributed over the southern states (United States of America), and as far north as in Nebraska and Iowa. However, during eighteen collecting trips into southern Texas and Louisiana, extending over the past twenty-three years, none of this species has been found in such areas as contained *Paratettix texanus* in large, and *Apotettix eurycephalus* in smaller, numbers. *A. arenosum* are found in considerable numbers in the region of Manhattan, Kansas, mainly in wooded areas, along the streams.

[1] Contribution 146 from the Department of Zoology, KANSAS STATE AGRICULTURAL EXPERIMENT STATION, Manhattan, Kansas.

[*] The cost of the accompanying color plate is paid by the GALTON AND MENDEL MEMORIAL FUND.

In superficial appearance the members of this species differ only slightly from those of *P. texanus* and *A. eurycephalus*, the real distinctions consisting of smaller, though taxonomically important, features. They are, perhaps, even more varied in the elementary and hybrid dominant color patterns of the pronota and femora of the posterior legs than are the southern species named above (see plate 1). They also vary greatly with respect to sizes, and there is the usual dimorphism of lengths of the pronota and wings, with occasional intermediates. Nothing has been done toward determining the causes of the variations in sizes, and in wings and pronota lengths or to determine the inheritance of these characteristics. With respect to these features they resemble *P. texanus* (NABOURS 1914), wherein the lengths of wings and pronota were found to be probably correlated with the length of the growth periods; they were preponderantly long in the spring and early summer when growth was more rapid, and short in the late summer and fall when the growth rate was slower.

The members of this species, as well as other varieties and species of grouse locusts, in the region of Manhattan, Kansas, and farther north, in contrast with southern ones, hibernate during the cold season, usually from some time in October till in April, with practically no activity except on unseasonably warm days. The duration of the period of hibernation appears to depend upon the latitude, or length of the season. They hibernate in tufts of grass, under stones, leaves, pieces of wood, etc. They have been observed to survive weather below 0° F. The mortality is usually high during the period of hibernation, due probably as much to desiccation as to low temperatures.

Even in the greenhouse, *A. arenosum*, except when continuously exposed to mercury vapor, or intense white light, do not breed at all during the period from September to April, inclusive. They are carried through the winter with great difficulty, and then the prolificacy of the few survivors seems to be much impaired. We have failed in varied and repeated efforts to produce, either indoors, outdoors, or in refrigerators, conditions tolerant for the kind of hibernation that would carry the pedigreed stock over the winter in good condition for further breeding the following spring. Therefore, mainly new and unknown stock, with females sometimes already mated to anonymous males, had to be brought in each year for the one, or two generations. Thus, although exhibiting great diversity of elementary and hybrid, dominant color patterns, and probably also many heritable form and size variations, this species affords genetic data slowly and very meagerly.

THE EFFECTS OF EXPOSURE TO CONTINUOUS LIGHT

Specimens of *A. arenosum* were kept under the continuous light of a mercury vapor lamp, at distances of 30 to 36 inches, with 12-mesh wire

intervening, for various lengths of time, during a period of about two years. While some of the results of this experiment were obscure, a few pairs of those exposed to the light produced offspring in the late autumn and early winter, as against no progeny from many controls living at approximately the same temperatures, under the ordinary conditions of the greenhouse. It was also ascertained that nymphs, while exposed to the mercury lamp, ate algae (their normal food) readily, were quite active, and grew rather rapidly to the imago, even in the late fall and winter, as compared with the controls which did not grow sufficiently to induce any ecdyses till late in April, or in May. Subsequently, CURTIS SABROSKY, working in our laboratory, has ascertained that quite intense light from 200-watt, filamentous, clear bulbs produces practically the same effects as does the mercury vapor lamp, the temperatures under both kinds of lights and for the controls, with comparable variations, running nearly the same.

THE COLOR PATTERNS

Thus far twenty-four distinct, elementary, dominant color patterns of the pronota and the femora of the posterior legs, and the generalized, mottled gray recessive, or "wild type" (+/+), have been employed in the experiments. The system of naming the patterns is similar to that used for *P. texanus* and *A. eurycephalus* (NABOURS 1929, Bibliography). Several of these patterns resemble, to a greater or less degree, some of those of the species named. However, there are considerable differences in the linkage relations of such similar patterns among these species, respectively, as a comparison discloses (see table 1 and the plate 1).

The following is an attempt to describe the elementary color patterns, all dominant except the first:

1. +/+. Mottled, or smooth gray. Comparable with the same pattern in most grouse locusts so far studied. Recessive to all other patterns yet examined in this species.

2. *B.* Pronotum chalky white, becoming uneven gray posteriorly. Reddish brown tipped pronotum and wings. Femora of anterior legs white; those of hind legs unevenly reddish to orange brown.

3. *Bil.* Creamy white, or yellow, bilateral lines extending from anterior carinae to humeral angle, and thence posteriorly about two-thirds of distance to end of pronotum.

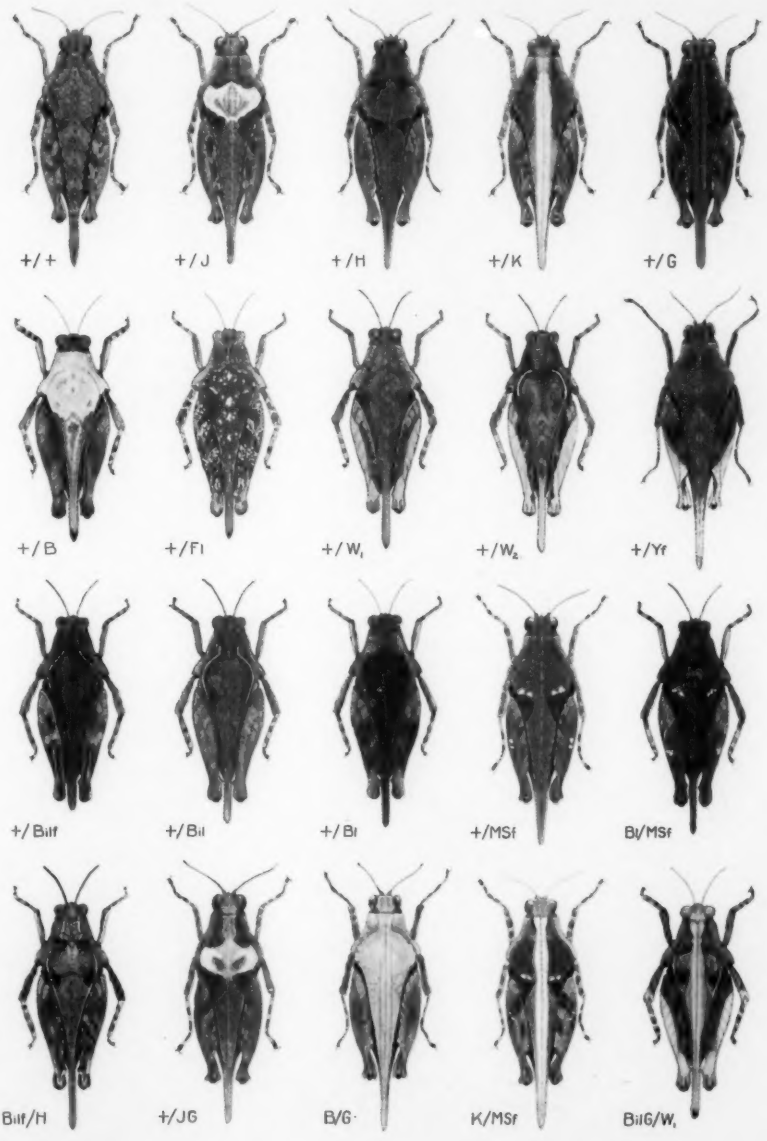
4. *Bilf.* Bilateral line, as in *Bil*, but heavier and extending from anterior end of pronotum. Lines also along upper posterior part of femora of hind legs.

5. *Bl.* Entire pronotum, upper parts of lateral lobes and posterior two-thirds of femora of jumping legs dense black.

LEGEND FOR PLATE 1

(*Acrydium arenosum* Burmeister)

The mottled gray, recessive, or "wild type" pattern (+/+) and fourteen of the dominant, elementary color patterns are shown in the first fourteen drawings, from left to right. The +/*M Sf* represents two elementary patterns, *M* and *Sf*, which are not indicated separately. The last seven drawings are representatives of the numerous hybrid complexes. S. FRED PRINCE made the first five, the tenth, fourteenth, seventeenth, eighteenth and nineteenth, and RUTH CLENCY made the other ten drawings.


+/+
+/J
+/H
+/K
+/G
+/B
+/Fl
+/W1
+/W2
+/Yf
+/Bilf
+/Bil
+/Bl
+/MSf
Bl/MSf
Bilf/H
+/JG
B/G
K/MSf
BilG/W1

6. *D*. Strong white spots above superior carinae near middle of hind femora.

7. *Fl*. Small, irregularly spaced white flecks over pronotum, lateral lobes, all femora, with general reddish brown background.

8. *G*. Tawny brown stripe along median pronotum.

9. *Gr*. Light, mottled gray over pronotum, lateral lobes and femora of jumping legs. Four small black spots distributed along median carina.

10. *H*. Light orange brown, oblong spot, the width of the pronotum, between the humeral angles.

11. *J*. Nearly clear white spot, with small area of mottled (+/+) pattern in center. Occupies the same area as *H*.

12. *K*. Gray white stripe along median pronotum.

13. *M*. One, or more, small, variable, white spots just posterior to the humeral angle, on each side of the pronotum.

14. *My*. A mahogany brown over posterior three-fourths of pronotum.

15. *Myfem*. Reddish brown, as in *T*, of jumping legs, and lines anterior to humeral angle, and reddish tip of pronotum.

16. *R*. Entire pronotum dull yellow. Femora of hind legs marked as if they carried pattern of W_2.

17. *Sf*. One or two small white spots on each femur of jumping legs, in nearly the same position as *D*.

18. *T*. Deep red brown extending all over pronotum and legs. Tips of wings and dorso-lateral sides of abdomen strongly tinged with red.

19. *W*. White to brown fine line along each femur of posterior legs.

20. W_1. Light brown or orange over most of the femora of the jumping legs, and extending as a fine line around the humeral angles, and affecting the tip of the pronotum.

21. W_2. Broad yellow stripe along each femur of hind legs, spreading out over distal two-fifths. Tip of pronotum yellow.

22. *Yf*. Light lemon yellow over distal fourth of each femur of jumping legs.

23. *Yfext*. Similar to *Yf*, but central part of yellow pattern extended.

Two other distinct patterns, one resembling *M*, though with outer edges of pattern extending anteriorly and posteriorly as a fine line along the lateral carinae, and another, (*N*), a dark, reddish brown nearly all over the pronotum, have been bred in small numbers. However, these two patterns do not enter into the data of this paper.

The effects or products of the combinations of the dominant elementary patterns vary greatly. Some combinations leave the constituents, respectively, distinct; others appear to blend, while a few of the dominant complexes are so entirely novel as to be fairly considered hybrid emergents. A long catalog of the manifold effects of patterns on each other and on the

TABLE 1

The following is a list of those dominant color patterns, and the recessive "wild type" which resemble one another in A. arenosum, P. texanus and A. eurycephalus (NABOURS *1929*).

A. arenosum		*P. texanus*		*A. eurycephalus*
+/+	mottled gray	+/+	recessive	+/+
B	white all over	*B*	dominant	O
Bl	black all over	Θ	dominant	Θ
D	white spot femora	*D*	dominant	..
G	brown stripe	*P*	dominant	*G*
H	brown spot	*H*	dominant	*H*
J	white spot	*J*	dominant	*Y*
K	white stripe	*K*	dominant	*K*
M	diagonal lines on pronotum	..	dominant	*M*
N	brown all over	*N*	dominant	..
R	yellow all over	..	dominant	*R*
Sf	(dom) specked femora	*sf/sf*	recessive	..
T	red all over	..	dominant	*T*
W_1	striped femora	..	dominant	*W*

results of three or more in combination has been made, but the few mentioned below, and the approximations of the drawings of the plate will have to suffice now. After some experience, the recorders are able, in most cases, even for individuals of considerable complexity, to ascertain by simple examination the composition with respect to the factors for component elementary patterns.

The elementary patterns *D* and *M* seem to retain their individuality, respectively, in whatever combinations found. *Bil* and *Bilf* are always comparatively distinct, except that the femoral lines of *Bilf* are obscured by *B*, *R*, W_1, W_2, *Yf* and *Yfext*, and *T* alters the appearance of *Bil* so that it becomes a light bar on the anterior pronotum. *Sf* is hardly distinguishable with *B* or W_1, but is quite distinct in combination with *W* and W_2. *K* shows as a very narrow, white stripe through the brown of *G*, reddish through *T*, and very white in connection with *B*.

The elementary pattern *G* may be identified in connection with all the patterns. It becomes chalky and lighter with *B*, blacker with *Bl*, very brown with *My*, reddish with *T*, and very little altered by *R*. *J* takes on almost any pattern nearby; it becomes whiter with *B*, darker with *Bl*, yellow with *R*, very red with *T*, and causes *G* to spread out where the two cross. *W* is obscured to some degree by almost any pattern on the femora. *R*, *B* and *Bl* are among the prominent patterns that greatly modify, but seldom obscure others.

THE RESULTS OF THE PAIRINGS OF FACTORS FOR ELEMENTARY DOMINANT COLOR PATTERNS

There were two kinds of matings: (1) That in which the parentage of either the male or the female, or both was on record, so that, at least, it

was known of the members of pairs of factors whether they had come in together, or separately from each of the parents; (2) that in which the relations of the members of pairs of factors with respect to parentage was not ascertainable. These were usually in the males and virgin females which had been collected in the field.

In the preparation of the data for table 2 showing percentages of crossing over of pairs of factors, the results from the matings of pedigreed individuals have been followed in estimating the position of segregates, or crossovers, of those of unknown ancestry. Since the pairs of factors, of the unpedigreed parents are so nearly the same in ratios of segregation and crossing over as those respectively of the pedigreed individuals, it is thought that few, if any, mistakes have been made. The following pair of linked genes taken at random, except for the larger numbers involved, indicates the general trend of the percentages to agree. In the matings, from the 476 pairings of the factors *My* and *W*, in the pedigreed males, there were 89 crossovers, or a frequency of 18.7 percent (not shown separately in the table). In the consideration of the offspring of the unpedigreed males bearing these factors, the females to which they were mated having been brought in as nymphs, or otherwise certified as virgin, the pairings were treated in the same way. This was irrespective of the lack of knowledge of the positions of *My* and *W* on the pair of chromosomes, whether they had both come from one parent, or the *My* from one, and the *W* from the other parent. From the 644 pairings of *My* and *W* in these unpedigreed males 148, or 23 percent, were crossovers. These have been added (see table 2) to the data of the pedigreed ones to make a total of 1120 pairings with 237 crossings over, or 21.2 percent, in the males.

Similarly treated, there were 571 pairings of *My* and *W* in the pedigreed females, with 114, or 20 percent, of crossing over, and 1027 pairings, with 142, or 13.8 percent, in the unpedigreed females. The data of the pedigreed and unpedigreed females together constitute 1598 pairings of *My* and *W*, with 256 or 16 percent. There were, therefore, 2718 pairings of *My* and *W* in both the males and females, with an average of 493, or 18.1 percent, of crossing over.

Not only in the example given of the factors *My* and *W* but throughout the table it may be observed that the crossing over of factors in the males, with few exceptions, has been comparable with that of the females. This is in striking contrast with the data from the experimental breeding, especially of *Apotettix eurycephalus*, in which there has been practically no crossing over of factors in the males, and *Paratettix texanus* in which the crossing over of the one factor, Θ, with the large group of extremely closely linked factors, or multiple allelomorphs, has been in the males very little over one-half as much as in the females.

TABLE 2

Showing segregation, or crossing over, between members of the pairs of factors for dominant color patterns in A. arenosum.[1]

FACTORS	MALES			FEMALES			MALES AND FEMALES		
	NUMBERS OF PAIRINGS	CROSS-OVERS	PERCENT OF CROSSOVER	NUMBERS OF PAIRINGS	CROSS-OVERS	PERCENT OF CROSSOVER	NUMBERS OF PAIRINGS	CROSS-OVERS	PERCENT OF CROSSOVER
B×*Bil*				13	0	0.0	13	0	0.0
Bilf				192	0	0.0	192	0	0.0
D	9	5	55.6				9	5	55.6
G	35	12	34.3	76	20	26.3	111	32	28.8
K	29	2	6.9	7	3	42.9	36	5	13.9
My	44	14	31.8	261	112	42.9	305	126	41.3
Sf	9	5	55.6				9	5	55.6
T				145	61	42.1	145	61	42.1
W	29	9	31.0	140	44	31.4	169	53	31.4
Bil×*Bl*	50	28	56.0	296	136	45.9	346	164	47.4
D	20	8	40.0				20	8	40.0
Fl				40	19	47.5	40	19	47.5
G	159	33	21.1	175	71	40.5	334	104	31.1
H	3	3	100.0				3	3	100.0
J	107	10	9.3	138	4	2.9	245	14	5.7
K	212	34	16.0	270	94	34.8	515	153	26.5
M				108	48	44.4	108	48	44.4
My	357	156	43.7	93	32	34.4	450	188	41.7
R	6	3	50.0				6	3	50.0
Sf	52	10	19.6	108	36	33.3	160	46	28.7
T				54	31	57.4	54	31	57.4
W	429	194	45.2	284	116	40.8	713	310	43.5
W_1	96	47	48.9	206	96	46.6	312	143	47.4
W_2	62	20	32.3				62	20	32.3
Yf	53	21	39.6	37	16	43.2	90	37	41.1
Yfext	167	71	42.5	37	17	45.9	204	88	43.1
Bilf×*Bl*	269	121	44.9	126	58	47.6	395	179	45.3
D	3	3	100.0				3	3	100.0
G	48	3	6.2	168	72	42.8	216	75	34.7
Gr	110	52	47.3	138	67	48.5	248	119	48.0
H	156	16	10.3				156	16	10.3
J	72	0	0.0	6	0	0.0	78	0	0.0
K	235	3	1.3	154	59	38.3	389	62	15.9
M	219	102	46.6	17	9	53.5	236	111	47.0
My	314	148	47.1	327	160	48.9	641	308	48.1
R	228	102	44.7				228	102	44.7
Sf	63	32	50.8	17	6	35.3	80	38	47.5

[1] Explanation: The factors are given in alphabetical order, as they were named when brought in from nature, or resembled those of *P. textanus* or *A. eurycephalus*, respectively. Each factor is shown in its pairings with all the others. Example: *B* with *Bil*, *Bilf*, *D*, etc. Then *Bil* with *Bl*, *D*, etc. Under the columns headed with "crossovers" and "percent of crossover" the term "or segregation" is implied.

TABLE 2 (*Continued*).

FACTORS	MALES			FEMALES			MALES AND FEMALES		
	NUMBERS OF PAIRINGS	CROSS-OVERS	PERCENT OF CROSSOVER	NUMBERS OF PAIRINGS	CROSS-OVERS	PERCENT OF CROSSOVER	NUMBERS OF PAIRINGS	CROSS-OVERS	PERCENT OF CROSSOVER
Bilf×*Bl*									
T				110	47	42.7	110	47	42.7
W	446	209	46.9	518	235	45.2	964	444	46.1
W_1	408	180	44.1	167	77	46.1	575	257	44.7
W_2	41	20	48.9	34	17	50.0	75	37	49.3
Yf				84	42	50.0	84	42	50.0
Bl×*G*	22	12	54.5	86	48	55.7	108	60	55.6
Gr	45	7	15.6				45	7	15.6
J	110	56	50.9	195	90	46.2	305	146	47.9
K	51	29	56.9	769	351	45.6	820	380	46.3
M	7	5	71.4				7	5	71.4
My				190	86	45.3	190	86	45.3
R	9	3	33.3				9	3	33.3
Sf	7	6	85.7				7	6	85.7
T				66	24	36.4	66	24	36.4
W	142	68	47.9	528	133	25.2	670	201	30.0
W_1	400	191	47.8	391	182	46.5	791	373	47.2
W_2	101	46	45.5				101	46	45.5
Yf				79	38	48.1	79	38	48.1
D×*G*	25	15	60.0	90	47	52.2	115	62	53.9
J				59	31	52.5	59	31	52.5
K	29	13	44.8	118	56	47.6	147	69	46.9
My	54	6	11.1	260	27	10.4	314	33	10.5
Sf	54	0	0.0	260	0	0.0	314	0	0.0
W	9	5	55.6	30	14	46.6	39	19	48.7
W_1	25	10	40.0	90	41	45.6	115	51	44.4
Fl×*G*	22	10	45.5				22	10	45.5
J	175	92	52.6	55	23	41.8	230	115	50.0
R	64	33	51.6	28	8	28.6	92	41	44.6
W	21	0	0.0				21	0	0.0
W_1	125	65	52.4	40	22	55.0	165	87	52.7
G×*Gr*				91	24	26.4	91	24	26.4
J	235	1	0.43	303	101	33.3	538	102	19.0
K	170	18	10.6	109	6	5.5	279	24	8.6
M	27	11	40.7	35	18	51.4	62	29	46.8
My	180	78	43.3	288	137	47.6	468	215	45.9
Sf	52	23	44.2	125	61	48.8	177	84	47.5
T	26	11	42.3	33	13	39.1	59	24	40.7
W	525	250	47.6	364	161	44.2	889	411	46.2
W_1	149	68	45.6	256	123	48.0	405	191	47.2
W_2	56	24	42.9				56	24	42.9
Yf	72	29	40.3	222	107	48.2	294	136	46.3
Gr×*J*				6	3	50.0	6	3	50.0
W_1	110	40	36.4	19	7	36.8	129	47	36.4

TABLE 2. (*Continued*).

FACTORS	MALES			FEMALES			MALES AND FEMALES		
	NUMBERS OF PAIRINGS	CROSS-OVERS	PERCENT OF CROSSOVER	NUMBERS OF PAIRINGS	CROSS-OVERS	PERCENT OF CROSSOVER	NUMBERS OF PAIRINGS	CROSS-OVERS	PERCENT OF CROSSOVER
H×*K*	199	4	2.0	103	7	6.8	302	11	3.6
M	41	24	58.5				41	24	58.5
My	148	75	50.7				148	75	50.7
W	188	91	48.4				188	91	48.4
W_1	3	3	100.0	42	17	40.5	45	20	44.4
W_2	103	44	42.7	103	43	41.7	206	87	42.2
Yf				42	16	38.1	42	16	38.1
J×*K*	208	21	10.1	236	91	38.6	444	112	25.2
M	21	5	23.8	20	8	40.0	41	13	31.7
My	316	142	44.9	542	265	48.9	858	407	47.4
Myfem	2	1	50.0				2	1	50.0
R	64	34	53.1	15	7	46.7	79	41	51.9
Sf	21	9	42.9	78	41	52.6	99	50	50.5
T	18	8	44.4	36	14	38.9	54	22	40.7
W	601	273	45.4	773	375	48.5	1374	648	47.2
W_1	592	283	47.8	468	229	48.9	1060	512	48.3
W_2	54	25	46.3	19	10	52.6	73	35	47.9
Yf	30	9	30.0	116	48	41.4	146	57	39.0
Yfext	13	6	46.2	55	25	45.5	68	31	45.6
K×*M*	72	32	44.4	32	13	40.6	104	45	43.3
My	474	207	43.7	804	357	44.4	1278	564	44.1
Myfem	32	16	50.0				32	16	50.0
R	183	84	45.9				183	84	45.9
Sf	29	13	44.8	145	66	45.5	174	79	45.4
T				79	42	53.2	79	42	53.2
W	977	430	44.0	1076	499	46.4	2053	929	45.3
W_1	176	97	51.1	249	116	46.5	425	213	50.1
W_2	134	55	41.0	146	66	45.2	280	121	43.2
Yf	12	6	50.0				12	6	50.0
Yfext				19	9	47.4	19	9	47.4
M×*My*	15	4	26.7	118	24	20.3	133	28	21.1
Sf	109	7	6.4	385	64	16.6	494	71	14.4
W	63	4	6.4	118	19	16.1	181	23	12.7
W_1				1	1	100.0	1	1	100.0
W_2	87	41	47.1	32	10	31.3	119	51	42.9
Yf				9	3	33.3	9	3	33.3
N	13	1	7.7				13	1	7.7
My×*R*	178	83	46.6	4	1	25.0	182	84	46.2
Sf	54	6	11.1	371	52	14.0	425	58	13.7
W	1120	237	21.2	1598	256	16.0	2718	493	18.1
W_1	79	37	46.8	132	55	41.7	211	92	43.6
W_2	16	4	25.0	10	3	30.0	26	7	26.9
Yf	58	17	29.3	116	56	48.3	174	73	42.0
Yfext	217	100	46.1	55	23	41.8	272	123	45.2

TABLE 2 (*Continued*).

FACTORS	MALES			FEMALES			MALES AND FEMALES		
	NUMBERS OF PAIRINGS	CROSS-OVERS	PERCENT OF CROSSOVER	NUMBERS OF PAIRINGS	CROSS-OVERS	PERCENT OF CROSSOVER	NUMBERS OF PAIRINGS	CROSS-OVERS	PERCENT OF CROSSOVER
Myfem×*W*	19	8	42.1				19	8	42.1
N×*W*	13	0	0.0				13	0	0.0
R×*W*	178	66	37.1	13	7	53.9	191	73	38.2
W_1	64	1	1.6	69	7	10.1	133	8	6.0
W_2	12	6	50.0				12	6	50.0
Sf×*W*	57	7	12.3	118	19	16.1	175	26	14.9
W_1	25	10	40.0	90	41	45.6	115	51	44.4
W_2				27	9	33.3	27	9	33.3
Yf	9	3	33.3				9	3	33.3
T×*W*	98	50	51.0	153	58	37.9	251	108	43.0
W×W_1	62	32	51.6	86	42	48.8	148	74	50.0
W_2	16	7	43.8	10	3	30.0	26	10	38.5
Yf	78	35	44.9	116	56	48.3	194	91	46.9
Yfext	217	87	40.1	55	15	27.3	272	102	37.5
W_1×*Yf*	33	4	12.1	42	5	11.9	75	9	12.0

Reference to the plate and descriptions of the color patterns in the text will give the symbols in the table greater meaning. It does not seem desirable to undertake to occupy the space that would be required to give the results of each mating in detail as was done in the account of the breeding of *A. eurycephalus* (NABOURS 1925).

THE DISTRIBUTION OF THE GENES FOR THE DOMINANT ELEMENTARY COLOR PATTERNS

The individuals of the species *Acrydium arenosum* have six pairs of autosomes, and of sex chromosomes, one in the male and a pair in the female. The chromosomes in this species are hardly distinguishable, with respect to number, size, form and arrangement, from those of *Paratettix texanus*, *Apotettix eurycephalus* and others of the grouse locusts (Tettigidae) so far studied (ROBERTSON 1915, 1930). No sex-linked factors have been noted. The genes responsible for the respective, elementary, dominant color patterns of *A. arenosum* are probably pretty well distributed among and along several, or all six of the pairs of the autosomes, as indicated by the data from the pairing of the genes presented in table 2. This is notably different from the situation in *A. eurycephalus* in which all thirteen genes for color

patterns and the lethal are closely linked on one (now known to be the smallest [NABOURS 1931, ROBERTSON 1931]) pair of the autosomes, and *P. texanus*, in which twenty-five or more genes for color patterns are extremely closely linked on one pair, and with only two or three others apparently on other pairs of autosomes.

There are at least three or four linkage groups, and probably more. The genes for the patterns, *B*, *Bil*, *Bilf*, *J*, *H*, *K* and *G* are quite easily located on one pair; *Bl*, *Gr* and *R* on another; W_2, *My*, *W*, *D*, *Sf*, and *M* on still another, while W_1 and *Yf* are probably on a fourth pair of chromosomes (see table 2).

Certain of the elementary patterns of *A. arenosum* closely resemble some of those of *P. texanus*, *A. eurycephalus*, and of other species, as indicated in table 1. However, there are considerable differences in the linkage relations among the pairs in the different species. For example, there is no crossing over between *J* and *K* in *P. texanus*, about 7 percent between *Y* (=*J*) and *K* in *A. eurycephalus*, and 25 percent between *J* and *K* in *A. arenosum*; complete linkage between *J* and *P* (=*G*) in *P. texanus*, 4 percent of crossing over between *Y* (=*J*) and *G* in *A. eurycephalus* (NABOURS 1929) and 19 percent between the genes for similar patterns in *A. arenosum*. It should be noted, however, that the patterns are not strictly alike, and they may be severally consequent upon entirely different, and never related, genes.

SUMMARY

1. The genes for twenty-four dominant, elementary color patterns of *A. arenosum* are apparently widely distributed among and along most of the six pairs of autosomes, three or more on the one pair, six, or more, on two others, respectively, and two or more on a fourth. The data might easily be interpreted so as to place genes for patterns on the other two pairs of autosomes. None is sex-linked.

2. Crossing over of factors for color patterns occurs in the males in about the same frequency as in the females.

3. Certain pairs of factors for dominant, elementary color patterns in *A. arenosum* differ markedly in linkage relations from those of similar patterns in *P. texanus*, and both of these vary considerably in crossing over values from comparable ones in *A. eurycephalus*.

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